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(54) Title: TARGETS FOR TUMOR GROWTH INHIBITION

(57) Abstract: The present invention relates to methods for treating cancers by manipulating a target gene expression by up-regulation, silencing and/or down-regulation of the gene, such as EGFR-RP, TRA1, MFGES, TNFSF13 and ZFP236, respectively. The methods are useful in treating cancers and/or inhibiting tumor growth by enhancing expression of a gene that is validated as a target such as ICT1030, for protein, peptide drug and gene therapy modalities; or by RNA interference to silence and/or down-regulate targets such as ICT1024, ICT1025 and ICT1031 and ICB1003 that are validated for antibody, small molecule and other inhibitor drug modalities.

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Targets for Tumor Growth Inhibition

This application claims priority to provisional applications 60/458,948 filed April 1, 2003, and 60/489,504, filed July 24, 2003, the specifications of which are hereby incorporated by reference in their entireties.

5 FIELD OF THE INVENTION

The present invention relates to methods for treating diseases by manipulating activity or expression of validated cancer drug targets, where the targets have been validated by methods manipulating target gene expression in animal disease models. More specifically, the invention relates to up-regulation, silencing, inhibition and/or down-regulation of targets
10 such as ICT1024, ICT1025, ICT1030, ICTB1031 and ICBT1003 that are validated using siRNA. The invention pertains to methods that are useful in treating cancers and/or inhibiting tumor growth by enhancing expression of a gene that is validated as target ICT1030 for protein, peptide and gene therapy drug modalities, or by RNA interference to silence and/or down-regulate genes that are validated as targets ICT1024, ICT1025, ICT1031
15 and ICT1003, for antibody, small molecule and other inhibitor drug modalities.

BACKGROUND OF THE INVENTION

Cancer or pre-cancerous growth generally refers to malignant tumors, rather than benign tumors. Benign tumor cells are similar to normal, surrounding cells. Treatment becomes necessary only when the tumors grow large enough to interfere with other organs.
20 Malignant tumors, by contrast, grow faster than benign tumors, and they penetrate and destroy local tissues. Some malignant tumors may spread throughout the body via blood or the lymphatic system. The unpredictable and uncontrolled growth makes malignant cancers dangerous, and fatal in many cases. These tumors are not morphologically typical of the original tissue and are not encapsulated. Malignant tumors commonly recur after surgical
25 removal.

Many human diseases are a result of proliferative cellular pathologies. Cancer or pre-cancerous growth is frequently a consequence of proliferative cellular pathologies and generally refers to malignant tumors, rather than benign tumors. Benign tumor cells are similar to normal, surrounding cells. Treatment becomes necessary only when the tumors
30 grow large enough to interfere with other organs. Malignant tumors, by contrast, grow faster than benign tumors, and they penetrate and destroy local tissues. Some malignant tumors

may spread throughout the body via blood or the lymphatic system, and their unpredictable and uncontrolled growth makes malignant cancers dangerous, and fatal in many cases. Such tumors are not morphologically typical of the original tissue and are not encapsulated.

Malignant tumors commonly recur after surgical removal. Accordingly, treatment of
5 proliferative diseases ordinarily targets proliferative cellular activities such as occur in malignant cancers or malignant tumors with a goal to intervene in the proliferative processes.

The inhibition or prevention of malignant growth is most effective at the early stage of the cancer development. It is important, therefore, to identify and validate molecular targets that play a role in proliferative processes and their induction and, in malignant
10 diseases in particular, early signs of tumor formation. A particular goal is to determine potent tumor growth or gene expression suppression elements or agents associated therewith. The development of such tumor growth and/or gene expression and therapeutic elements or agents involves an understanding of the genetic control mechanisms for cell division and differentiation, particularly in connection with tumorigenesis. Unfortunately, the number of
15 established protein targets that are suitable for intervention in proliferative disease is limiting. Of the small number of established targets, such as growth factors like EGF and its receptor, few, if any, permit adequate intervention in proliferative diseases such as malignant cancer and psoriasis.

Moreover, it has proven difficult to identify better targets for intervention in cellular
20 proliferative pathologies. Large numbers of genes and proteins exist within the human genome and many of these genes and proteins, as well as post-translationally modified forms of the proteins, correlate with cellular proliferative pathologies. Of these many genes, proteins, and post-translationally modified proteins, only a few specific factors can be targeted to effectively intervene in cellular proliferative pathologies. Therefore, identification
25 of these specific factors is needed. In addition to a need to identify specific genes, proteins, and post-translationally modified proteins to target to intervene in proliferative cellular pathologies, another problem is a need to confirm that the targeted factor indeed provides effective intervention within the active pathology within active pathological tissues. Unfortunately, proliferation of cells in cell culture conditions shows many factors can be
30 targeted but most ultimately do not prove effective as intervention targets in active pathological tissues. Consequently, accurate identification of targets for effective intervention in proliferative cellular pathologies requires study of active pathological tissues such as in animal models of human disease.

Accordingly, treatment ordinarily targets malignant cancers or malignant tumors. The intervention of malignant growth is most effective at the early stage of the cancer development. It is thus exceedingly important to identify and validate a target for early signs of tumor formation and to determine potent tumor growth or gene expression suppression elements or agents associated therewith. The development of such tumor growth and/or gene expression and therapeutic elements or agents involves an understanding of the genetic control mechanisms for cell division and differentiation, particularly in connection with tumorigenesis.

RNA interference (RNAi) is a post-transcriptional process where the double-stranded RNA (dsRNA) inhibits gene expression in a sequence specific fashion. The RNAi process occurs in at least two steps: in first step, the longer dsRNA is cleaved by an endogenous ribonuclease into shorter, less than 100-, 50-, 30-, 23-, or 21-nucleotide-long dsRNAs, termed "small interfering RNAs" or siRNAs. In second step, the smaller siRNAs mediate the degradation of the target mRNA molecule. This RNAi effect can be achieved by introducing either longer dsRNA or shorter siRNA to the target sequence within cells. It is also demonstrated that RNAi effect can be achieved by introducing plasmids that generate dsRNA complementary to target gene.

The RNAi have been successfully used in gene function determination in *Drosophila* (Kennerdell et al. (2000) *Nature Biotech* 18: 896-898; Worby et al. (2001) *Sci STKE* Aug 14, 2001(95):PL1; Schmid et al. (2002) *Trends Neurosci* 25(2):71-74; Hammond et al. (2000). *Nature*, 404: 293-298), *C. elegans* (Tabara et al. (1998) *Science* 282: 430-431; Kamath et al. (2000) *Genome Biology* 2: 2.1-2.10; Grishok et al. (2000) *Science* 287: 2494-2497), and Zebrafish (Kennerdell et al. (2000) *Nature Biotech* 18: 896-898). In those model organisms, it has been reported that both the chemically synthesized shorter siRNA or in vitro transcribed longer dsRNA can effectively inhibit target gene expression. There are increasing reports on successfully achieved RNAi effects in non-human mammalian and human cell cultures (Manche et al. (1992). *Mol. Cell. Biol.* 12:5238-5248; Minks et al. (1979). *J. Biol. Chem.* 254:10180-10183; Yang et al. (2001) *Mol. Cell. Biol.* 21(22):7807-7816; Paddison et al. (2002). *Proc. Natl. Acad. Sci. USA* 99(3):1443-1448; Elbashir et al. (2001) *Genes Dev* 15(2):188-200; Elbashir et al. (2001) *Nature* 411: 494-498; Caplen et al. (2001) *Proc. Natl. Acad. Sci. USA* 98: 9746-9747; Holen et al. (2002) *Nucleic Acids Research* 30(8):1757-1766; Elbashir et al. (2001) *EMBO J* 20: 6877-6888; Jarvis et al. (2001) *TechNotes* 8(5): 3-5; Brown et al. (2002) *TechNotes* 9(1): 3-5; Brummelkamp et al. (2002)

Science 296:550-553; Lee et al. (2002) *Nature Biotechnol.* 20:500-505; Miyagishi et al. (2002) *Nature Biotechnol.* 20:497-500; Paddison et al. (2002) *Genes & Dev.* 16:948-958; Paul et al. (2002) *Nature Biotechnol.* 20:505-508; Sui et al. (2002) *Proc. Natl. Acad. Sci. USA* 99(6):5515-5520; Yu et al. (2002) *Proc. Natl. Acad. Sci. USA* 99(9):6047-6052).

- 5 **EGFR-RP (Validated Target ICT1024):** Homo sapiens Epithelial growth factor receptor-related protein, EGFR-RP or EGFR-RS is published GenBank accession nos. are AK026010, NM_022450, BC014425, AK056708 and M99624.

10 All eukaryotic cells contain elaborate systems of internal membranes which set up various membrane-enclosed compartments within the cell. The plasma membrane serves as the interface between the machinery in the interior of the cell and the extracellular fluid (ECF) that bathes all cells. Cell membranes are built from lipids and proteins. The lipids in the plasma membrane are chiefly phospholipids like phosphatidyl ethanolamine and cholesterol. Phospholipids are amphiphilic with the hydrocarbon tail of the molecule being hydrophobic; its polar head hydrophilic. As the plasma membrane faces watery solutions on both sides, its phospholipids accommodate this by forming a phospholipid bilayer with the hydrophobic tails facing each other. Many of the proteins associated with the plasma membrane are tightly bound to it. Some are attached to lipids in the bilayer, and others are transmembrane proteins - the polypeptide chain actually traverses the lipid bilayer.

20 All membrane proteins have a specific upside-down or right-side-up orientation in the bilayer. Some proteins are anchored to the membrane by ionic interactions between residues with positively charged side chains and negatively charged lipid head groups since biological membranes tend to have a net negative charge. Other proteins are anchored by post-synthetic attachment of a hydrocarbon chain such as myristoyl, palmitoyl, farnesyl or geranyl-geranyl, or a lipid such as glycosylphosphatidylinositol (GPI) which confines them in regions close to their protein partners. Other proteins are anchored to the surface by ionic contacts. The term monotopic or peripheral membrane protein refers to proteins that have a fairly shallow penetration of the membrane surface. Many peripheral proteins can be released from the membrane by increasing the ionic strength of the solution. A second category of membrane proteins is integral or transmembrane bitopic or multitopic proteins. These proteins can only be released from the membrane by bilayer disruption with detergents.

30 Many transmembrane proteins that are structurally related are also functionally related. For example, the EGF (epidermal growth factor receptor) and the insulin receptor fall into a family of growth factor receptors which have very large disulfide-rich extracellular and a

tyrosine kinase intracellular domains connected by a single-transmembrane helix. Most members of this family are monomers and binding of ligand induces dimerization and activation of the intracellular tyrosine kinase domain. The insulin receptor is a dimer in its non-ligand bound state and it is possible that in this case the binding of insulin changes the intersubunit orientation of the monomers, allowing for activation.

Another important family of transmembrane proteins is the seven transmembrane family of G proteins (guanine nucleotide binding proteins) coupled receptors. These receptors are the most abundant class of receptors in mammalian cells and mediate an extremely diverse range of signals into the cell, from light (rhodopsin) to neurotransmitters (muscarinic or adrenergic receptors) to sex-related signals (oxytocin). Although their ligand activators are diverse, these receptors all couple to G proteins to transduce their signal. Structurally, they are similar in having seven transmembrane loops in a defined topology. In contrast to the growth factor receptor family, these proteins have relatively small extramembrane loops.

Integral membrane proteins that transport species such as nutrients and ions must be able to shield their ligands from the surrounding hydrocarbon interior. Thus, these proteins are much larger than the signal transduction proteins mentioned above, and often contain several subunits. An example of this class is the 12 membrane spanning family belonging to transporters, such as GLUT1 and antibiotics. A newly identified family of integral membrane proteins, Rhomboid family, is exemplified by the rhomboid (RHO) protein from *Drosophila melanogaster*, a developmental regulator involved in epidermal growth factor (EGF)-dependent signaling pathways (1, 2, 3). Not only were homologs of rhomboid detected in prokaryotes and eukaryotes, but the pattern of sequence conservation in this family appeared uncharacteristic of nonenzymatic membrane proteins, such as transporters (4,5). Specifically, several polar amino-acid residues are conserved in nearly all members of the rhomboid family, suggesting the possibility of an enzymatic activity. As three of these conserved residues were histidines, it appears that rhomboid-family proteins may function as metal-dependent membrane proteases (5, 6). Recently, however, it has been shown that RHO cleaves a transmembrane helix (TMH) in the membrane-bound precursor of the TGF α -like growth factor Spitz, enabling the released Spitz to activate the EGF receptor, and that a conserved serine and a conserved histidine in RHO are essential for this cleavage (7, 8). Thus, it appears that rhomboid-family proteins are a distinct group of intramembrane serine proteases. Altogether, the genome of *Drosophila* encodes seven RHO paralogs (now designated RHO1-7, with the original rhomboid becoming RHO-1), at least three of which

are involved in distinct EGF-dependent pathways, apparently through proteolytic activation of diverse ligands of the EGF receptor.

One human gene sharing homology with multiple cDNA sequences (Accession No. AK026010, NM_022450, Z69719, AK056708, BC014425, M99624) has been annotated as an ortholog of mouse epidermal growth factor receptor related sequence (EGFR-RS), hypothetical protein similar to epidermal growth factor receptor-related protein, human epidermal growth factor receptor-related gene, and lately human rhomboid family 1. The cDNA sequences AK026010, BC014425 and NM_022450 encode the same 855 amino acid protein (Accession No. BAB15318, AAH14425 and AAA02490). However, the biological activity of this protein presently is unknown.

TRA1 (Validated Target ICT1025): Homo sapiens Tumor rejection antigen, TRA1 or heat shock protein gp96 or grp94 is published with GenBank accession nos. NM_003299, AK025459, BC009195, AY040226, X15187 and AF087988. See also, U.S. Publication Nos. 2003/0215874; 2003/0054996; and 2002/0160496.

One of the targets selected with Efficacy-First, tumor rejection antigen-1 (TRA-1), was found to have increased expression in tumors induced to accelerated growth. TRA-1, also known as glucose-regulated protein 94 (grp94), gp96, endoplasmic precursor and other names, was first described as a molecular chaperone [Hartl FU. (1996) Molecular chaperones in cellular protein folding. *Nature* 381(6583):571-9] with important roles in endoplasmic reticulum related to nuclear signaling, protein folding, sorting and secretion [Nicchitta, C.V. (1998): Biochemical, cell biological and immunological issues surrounding the endoplasmic reticulum chaperone GRP94/gp96. *Current Opinion in Immunology*, 10:103-109.]. In addition, it exerts a specific protection against Ca²⁺ depletion stress and is involved in antigen presentation [Tamura, Y. P. Peng, K. Liu, M. Daou, P.K. Srivastava, 1997: Immunotherapy of tumors with autologous tumor-derived heat shock protein preparation. *Science*, 278:117-120]. Furthermore, it also has an important role in tumorigenicity [Udono H, Levey DL, Srivastava PK. (1994) Cellular requirements for tumor-specific immunity elicited by heat shock proteins: tumor rejection antigen gp96 primes CD8⁺T cells in vivo. *Proc Natl Acad Sci USA* 91: 3077-3081.]. Menoret et al. [Menoret A, Meflah K, Le Pendu J. (1994) Expression of the 100 kDa glucose-regulated protein (GRP100/endoplasmic) is associated with tumorigenicity in a model of rat colon adenocarcinoma. *Int J Cancer* 56: 400-405] reported that there was an overexpression of TRA-1 in a model of rat colon adenocarcinoma. Gazit et al. [Gadi Gazit, Jun lu, Amy S.Lee. (1999) De-regulation of GRP stress protein

- expression in human breast cancer cell lines. *Breast Cancer Research and Treatment* 54: 135-146.] found out there was a 3-5 fold increase in the level of TRA-1 protein was observed in five human breast cancer lines as compared to the normal human mammary lines. Cai et al. [Cai JW, Henderson BW, Shen JW, et al (1993) Induction of glucose-regulated proteins during growth of murine tumor. *J Cell Physiol* 154: 229-237] found through studies during growth of tumors that the level of the TRA-1 is increased, correlating with the size of the tumor. Elevated level of TRA-1 has been implicated to protect neoplastic cells and tumors against cytotoxic T-lymphocyte mediated cytotoxicity and protected tissue culture cells against adverse physiological conditions [Sugawara S, Takeda K, Lee A, et al. (1993) Suppression of stress protein GRP78 induction in tumor B/C10ME eliminates resistance to cell mediated cytotoxicity. *Cancer Research*. 53: 6001-6005]. Public domain databases reveal that TRA-1 is over-expressed in many human cancer tissues including prostate, mammary, brain, stomach, and soft tissue tumors. Overexpression, antisense and ribozyme approaches in tissue culture system directly showed that TRA-1 could protect cells against cell death [Little E, Ramakrishnan M, Roy B, et al. (1994) The glucose-regulated proteins (GRP78 and GRP94): Functions, gene regulation, and applications. *Crit Rev Eukaryot Gene Expr* 4: 1-18, Garrido C, Gurbuxani S, Ravagnan L, Kroemer G. (2001). Heat shock proteins: endogenous modulators of apoptotic cell death. *Biochem Biophys Res Commun*. 286(3):433-42., Ramachandra K. Reddy, et al. (1999). The endoplasmic reticulum chaperone glycoprotein GRP94 with Ca²⁺-binding and antiapoptotic properties is a novel proteolytic target of calpain during etoposide-induced apoptosis. *J. Biol. Chem* 274: 28476-28483]. These anti-apoptosis effects of TRA-1 are associated with induction in neoplastic cells and may lead to cancer progression and chemotherapy resistance. Although normally confined to the ER, TRA-1 has been shown to escape to KDEL-mediated retention system in several cell types. For instance, a significant fraction of TRA-1 is secreted to the extracellular space by hepatocytes and exocrine pancreatic cells, via the normal secretory pathway. In several tumor cell lines TRA-1 is detectable as an outer surface protein [Altmeyer A, Maki RG, Feldweg AM, Heike M, Protopopov VP, Masur SK, Srivastava PK (1996). Tumor-specific cell surface expression of the-KDEL containing, endoplasmic reticular heat shock protein gp96. *Int. J. Cancer* 22;69(4):340-9.].

TRA-1 has been shown to chaperone a broad array of peptides, including those derived from normal proteins as well as from foreign and altered proteins present in cancer or virus-infected cells. Thus, tumor-derived TRA-1 carries tumor antigenic peptides, and its

preparations from virus-infected cells carry viral epitopes. Although TRA-1 is normally intracellular, necrotic cells release TRA-1 peptide complexes, which are taken up by scavenging antigen-presenting cells. Presentation of the peptides on the surface of these cells leads to stimulation of T lymphocytes and a pro-inflammatory response.

5 Complexes of TRA-1 with peptides, whether isolated from cells or reconstituted in vitro, have been demonstrated to serve as effective vaccines, producing anti-tumor immune responses in animals and in man [Tamura, Y. P. Peng, K. Liu, M. Daou, P.K. Srivastava, 1997: Immunotherapy of tumors with autologous tumor-derived heat shock protein preparation. *Science*, 278:117-120.]. Oncophage is a vaccine made from individual patients' tumors. Patients have surgery to remove part or all of the cancerous tissue, and a portion of this tissue is shipped overnight to Antigenics' manufacturing facility in Massachusetts. The Oncophage clinical studies in several cancers including pancreatic, melanoma, kidney, colorectal, gastric, and non-Hodgkin's lymphoma have yielded very promising results. Their analysis provides a strong indication that antigen presentation by TRA-1 can induce an immune response in patients and clinical responses. With melanoma or colorectal cancer in one study, 10 out of 39 melanoma patients responded clinically to Oncophage treatment, including two patients whose cancer disappeared completely for more than two years. Of the 24 melanoma patients who were evaluated for immune response, 10 demonstrated increased antimelanoma T-cell activity. In colorectal cancer patients, a T-cell response was observed in 17 out of 29 patients, and seemed to be correlated with survival. The mechanism by which Oncophage induces immune response in melanoma and colorectal cancer was determined to be the same—confirming a wealth of preclinical and early clinical data demonstrating that this mechanism is virtually identical in all cancers and species tested to date.

25 **MFGE8 (Validated Target ICT1030):** Homo sapiens milk fat globule-EGF factor 8 protein (MFGE8) or breast epithelial BA46 antigen is published under GenBank accession nos. is NM_005928 and BC003610. US patent no. 6,339,066 B1 describes aspects of MFGE8 related molecules such as 'protein kinase C-eta' (PKC- η).

30 **TNFSF13 (Validated Target ICT1031):** Homo sapiens Tumor necrosis factor ligand super family member 13 (TNFSF13) is published GenBank accession nos. are AK090698 and O75888. Several international patent applications describe aspects of TNFSF13 related molecules such as APRIL (A proliferation-inducing ligand), TALL-2 (TNF-and APOL-related leukocyte expressed ligand 2), and TRDL-1 (TNF-related death ligand-1) (see, for example, WO 99/12965 and WO 01/60397).

ZFP236 (Validated Target ICT1003): Homo sapiens zinc finger protein 236 (ZFP236) is published under GenBank accession no. AK000847.

SUMMARY OF THE INVENTION

5 The invention provides methods for treating diseases, such as cancers, by up-regulation, silencing, or down-regulation of a validated target gene expression, by nucleic acid interaction, by introducing RNA interference or other agents, such as antibodies, soluble receptors, small molecule inhibitors, and the like, to modulate activity of a validated drug target, and as a result inhibit tumor growth.

10 One aspect of the invention provides methods for treating a disease, for example, a cancer or a precancerous growth, in a mammal associated with undesirable expression of a target ICT1030 gene, comprising applying a nucleic acid composition that interacts with the target ICT1030 DNA or RNA, wherein the nucleic acid composition is capable of enhancing expression of the target ICT1030 gene when introduced into a tissue of the mammal.

15 According to another aspect of the invention, nucleic acid molecules are introduced into tissues, including breast tissue, colon tissue, prostate tissue, skin tissue, bone tissue, parotid gland tissue, pancreatic tissue, kidney tissue, uterine cervix tissue, lung tissue, lymph node tissue, or ovarian tissue, wherein the nucleic acid molecule is a decoy molecule, a decoy DNA, a double stranded DNA, a single-stranded DNA, a complexed DNA, an encapsulated
20 DNA, a viral DNA, a plasmid DNA, a naked RNA, an encapsulated RNA, a viral RNA, a double stranded RNA, a molecule capable of enhancing expression of the target ICT1030 gene, or combinations thereof.

25 In the another aspect, the invention provides methods for inhibiting cancer or precancerous growth in a mammalian tissue, comprising contacting the tissue with an enhancer that interacts with the target ICT1030 DNA or RNA and thereby enhances the target ICT1030 gene expression.

30 In the another aspect, the invention provides methods for inhibiting cancer or precancerous growth in a mammalian tissue, comprising contacting the tissue with an enhancer that interacts with the target ICT1030 peptide and thereby enhances the target ICT1030 gene expression, wherein the tissue is breast tissue, colon tissue, prostate tissue, skin tissue, bone tissue, parotid gland tissue, pancreatic tissue, kidney tissue, uterine cervix tissue, lymph node tissue, or ovarian tissue, wherein the enhancer is a nucleic acid molecule, a decoy molecule, a decoy DNA, a double stranded DNA, a single-stranded DNA, a

complexed DNA, an encapsulated DNA, a viral DNA, a plasmid DNA, a naked RNA, an encapsulated RNA, a viral RNA, a double stranded RNA, a molecule capable of enhancing the target ICT1030 or combinations thereof.

Another aspect of the invention provides methods of administering nucleic acid to a patient in need thereof, wherein the nucleic acid molecule is delivered in the form of a naked oligonucleotide or a vector, wherein the nucleic acid interacts with the target ICT1030 gene.

Yet another aspect of the invention provides methods of administering nucleic acid to a patient in need thereof, wherein the nucleic acid molecule is delivered in the form of a naked oligonucleotide or a vector, wherein the nucleic acid interacts with the target ICT1030 gene, wherein the nucleic acid is delivered as a vector, wherein the vector is a plasmid, cosmid, bacteriophage, or a virus, for example, a retrovirus or an adenovirus based vector.

Still another aspect of the invention provides methods of enhancing in vivo expression of a gene by administering a vector to a patient in need thereof, wherein the vector containing target ICT1030 gene, wherein the nucleic acid interacts with the target ICT1030 gene expression, wherein the nucleic acid enhances the target ICT1030 gene expression in a mammalian cell, for example, a human cell.

According an aspect of the invention, the target ICT1030 gene, as described herein, comprises a polynucleotide selected from the group consisting of: a) a polynucleotide encoding the polypeptide set forth in SEQ ID NO:2; b) a polynucleotide set forth in SEQ ID NO:1; and SEQ ID NO:3; or c) a polynucleotide having at least about 90% sequence identity to the polynucleotide of a) or b).

In another aspect, the invention provides methods for treating a disease, for example, a cancer or a precancerous growth, in a mammal associated with undesirable expression of a target ICT 1024 or ICT 1025 or ICT 1003 or ICT1031 gene, comprising applying a nucleic acid composition containing an inhibitor that interacts with the target ICT 1024 or ICT 1025 or ICT 1003 or ICT1031 DNA or RNA, wherein the nucleic acid composition is capable of reducing expression of the target ICT 1024 or ICT 1025 or ICT 1003 or ICT1031 gene when introduced into a tissue of the mammal.

According to another aspect of the invention, nucleic acid molecules are introduced into tissues, including breast tissue, colon tissue, prostate tissue, skin tissue, bone tissue, parotid gland tissue, pancreatic tissue, kidney tissue, uterine cervix tissue, lung tissue, lymph node tissue, or ovarian tissue.

According to another aspect, the invention provides methods for treating a disease, for example, a cancer or a precancerous growth, in a mammal, comprising applying a nucleic acid composition containing an inhibitor that interacts with the target ICT 1024 or ICT 1025 or ICT 1003 or ICT1031 DNA or RNA, wherein the inhibitor is a siRNA, an RNAi, a shRNA,
5 an antisense RNA, an antisense DNA, a decoy molecule, a decoy DNA, a double stranded DNA, a single-stranded DNA, a complexed DNA, an encapsulated DNA, a viral DNA, a plasmid DNA, a naked RNA, an encapsulated RNA, a viral RNA, a double stranded RNA, a molecule capable of generating RNA interference, or combinations thereof.

In another aspect, the invention provides methods for inhibiting cancer or
10 precancerous growth in a mammalian tissue, comprising contacting the tissue with an inhibitor that interacts with a target ICT 1024 or ICT 1025 or ICT 1003 or ICT1031 protein, DNA or RNA and thereby reduces target ICT 1024 or ICT 1025 or ICT 1003 or ICT1031 activity or gene expression.

Yet in another aspect, the invention provides methods for inhibiting cancer or
15 precancerous growth in a mammalian tissue, wherein the tissue is a breast tissue, colon tissue, prostate tissue, skin tissue, bone tissue, parotid gland tissue, pancreatic tissue, kidney tissue, uterine cervix tissue, lung tissue, lymph node tissue, or ovarian tissue.

Still in another aspect, the invention provides methods for inhibiting cancer or precancerous growth in a mammalian tissue, comprising contacting the tissue with an
20 inhibitor, wherein the inhibitor is a siRNA, an RNAi, a shRNA, an antisense RNA, an antisense DNA, a decoy molecule, a decoy DNA, a double stranded DNA, a single-stranded DNA, a complexed DNA, an encapsulated DNA, a viral DNA, a plasmid DNA, a naked RNA, an encapsulated RNA, a viral RNA, a double stranded RNA, a molecule capable of generating RNA interference, or combinations thereof.

25 According an aspect of the invention, the target ICT1031 gene, as described herein, comprises a polynucleotide selected from the group consisting of: a) a polynucleotide encoding the polypeptide set forth in SEQ ID NO:5; b) a polynucleotide set forth in SEQ ID NO:4; and c) a polynucleotide having at least about 90% sequence identity to the polynucleotide of a) or b).

30 According an aspect of the invention, the target ICT1003 gene, as described herein, comprises a polynucleotide selected from the group consisting of: a) a polynucleotide encoding the polypeptide set forth in SEQ ID NO:7; b) a polynucleotide set forth in SEQ ID

NO:6 or SEQ ID NO:8; and c) a polynucleotide having at least about 90% sequence identity to the polynucleotide of a) or b).

In another aspect, the invention provides methods of administering siRNA to a patient in need thereof, wherein the siRNA molecule is delivered in the form of an oligonucleotide in a naked form or in a formulation or a vector, wherein the siRNA interacts with a target ICT 1024 or ICT 1025 or ICT 1003 or ICT1031 gene or a target ICT 1024 or ICT 1025 or ICT 1003 or ICT1031 mRNA transcript, wherein the siRNA is delivered as a vector, wherein the vector is a plasmid, cosmid, bacteriophage, or a virus, for example, a retrovirus or an adenovirus based vector.

Another aspect of the inventions provides methods of blocking in vivo expression of a target ICT 1024 or ICT 1025 or ICT 1003 or ICT1031 gene by administering a vector to a patient in need thereof, wherein the vector containing a target ICT 1024 or ICT 1025 or ICT 1003 or ICT1031 siRNA, wherein the siRNA interferes with target ICT 1024 or ICT 1025 or ICT 1003 or ICT1031 gene expression, for example, the siRNA causes post-transcriptional silencing of the target ICT 1024 or ICT 1025 or ICT 1003 or ICT1031 gene in a mammalian cell such as a human cell.

In another aspect, the invention provides methods for treating a disease, for example, a cancer or a precancerous growth, in a mammal associated with undesirable expression of a target ICT 1024 or ICT 1025 or ICT 1003 or ICT1031 gene, comprising applying a nucleic acid composition containing an inhibitor that interacts with the target ICT 1024 or ICT 1025 or ICT 1003 or ICT1031 DNA or RNA, wherein the nucleic acid composition is capable of reducing expression of the target ICT 1024 or ICT 1025 or ICT 1003 or ICT1031 gene when introduced into a tissue of the mammal.

According to another aspect, the invention provides methods for treating a disease, for example, a cancer or a precancerous growth, in a mammal, comprising applying a nucleic acid composition containing an inhibitor that interacts with the target ICT1003 DNA or RNA, wherein the inhibitor is a siRNA, an RNAi, a shRNA, an antisense RNA, an antisense DNA, a decoy molecule, a decoy DNA, a double stranded DNA, a single-stranded DNA, a complexed DNA, an encapsulated DNA, a viral DNA, a plasmid DNA, a naked RNA, an encapsulated RNA, a viral RNA, a double stranded RNA, a molecule capable of generating RNA interference, or combinations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows that the targets identified by the Efficacy-First Discovery™ method are different from those identified using a conventional approach. Expression changes of the targets are due to perturbation of delivered genes and disease process dynamic. They are better fit for drug discovery.

Figure 2 indicates that among a total of 156 selected targets, 111 were known based on UniGene database annotations and 45 were unknown novel targets. Within the known targets, 87% are tumor related. If the same ratio holds the truth, we expect more than 35 targets are novel tumor targets. In addition, the hits also belong to several tumorigenesis pathways.

Figure 3 shows validated two novel targets: ICT1030 and ICT1031. Among the selected targets tested with siRNA knockdown in vivo, 2 targets (ICT1030 and ICT1031) were validated with n=8 (8 tumors per cohort). Two proteins are cell surface factors with totally opposite effects. ICT1030 knockdown by specific siRNA resulted in tumor growth enhancement, versus ICT1031 knockdown triggered tumor growth inhibition. So that the former may be protein or gene therapy drug and the later could be an antibody or small molecular drug target.

Figure 4 shows one of the selected targets, ICT1003, which was tested with siRNA knockdown in vivo (8 tumors per cohort). The target ICT1003 is a novel zinc finger protein and may represent a transcription factor. ICT1003 knockdown by specific siRNA resulted in tumor growth inhibition. So that the protein could be a siRNA drug target or small molecular drug target.

Figure 5 shows a novel target, ICT1024, Accession No. AK026010, NM_022450, human growth factor receptor-related protein, EGFR-RP, or EGFR-RS, has been first identified by Efficacy-First discovery method due to its highly up-regulated expression in bFGF treated tumor (MDA-MB-435 cell) tissues. siRNA knockdown of this gene in the cell culture (MDA-MB-435 cell) study resulted activated apoptosis status. SiRNA knockdown of this gene in the xenograft tumors (MDA-MB-435 cell) resulted in tumor growth inhibition. The gene over expressed in several human tumors including breast and prostate cancer. The coded protein of this gene has a Rhomboid domain and a transmembrane domain.

Figure 6 shows a novel target, ICT1025, NM_003299, human tumor rejection antigen, TRA1, HSP gp96, has been first identified by Efficacy-First discovery method due to its highly up-regulated expression in bFGF treated tumor (MDA-MB-435 cell) tissues. siRNA

knockdown of this gene in the cell culture (MDA-MB-435 cell) study resulted activated apoptosis status. siRNA knockdown of this gene in the xenograft tumors (MDA-MB-435 cell) resulted in tumor growth inhibition. The gene over expressed in several human tumors including brain, breast, colon, ovary and prostate cancer. The coded protein of this gene has
5 a ATPase domain of HSP90 and the Hsp90 protein.

Figure 7 shows that the ICT1024 siRNA Design: two 21 nt sequences from ICT1024 were selected as the targets for RNAi-mediated knockdown of ICT1024 gene expression. (SEQ ID NO: 25 and SEQ ID NO: 26)

Figure 8 shows that ICT1024 siRNA duplexes inhibit growth of MDA-MB-435 cell
10 formed xenograft tumor on nude mice.

Figure 9 shows that ICT1024 siRNA duplexes induce apoptosis activity in a MDA-MB-435 cell culture assay.

Figure 10 shows that expression of ICT1024 in breast tumor tissue has significantly positive correlation with other cancer genes, based on the SAGE/microarray analysis.

Figure 11 shows that ICT1024 is highly up regulated in all Stage I Breast Tumor
15 samples (100%), based on Gene Logic GeneExpress analysis.

Figure 12 shows that ICT1024 protein has significant structural homology to other rhomboid proteins from various organisms, such as yeast, bacteria and plant. (SEQ ID NOs: 27 - 35)

Figure 13 shows that ICT1024 is a novel human protein and only shares structural
20 homology with other human rhomboid proteins in the C-terminal domain. (SEQ ID NO: 37 (ICT1024); SEQ ID NO:38 (HRhomboid 2); SEQ ID NO:39 (HRhomboid 3); SEQ ID NO:40 (HRhomboid 4); SEQ ID NO:41 (HRhomboid 5) and SEQ ID NO:42 (HRhomboid 6).

Figure 14 shows that ICT1024 does not share DNA or protein sequence homology
25 with other human rhomboid proteins. The siRNA targeted sequences is uniquely designed for ICT1024 protein.

Figure 15 shows that the cellular location and topology predication of ICT1024
protein based on multiple hydrophobicity analyses. At least 6 transmembrane domains were predicted with one additionally questioned 7th domains. However, the N-terminal portion of
30 the protein has a large region (1-400 or 1-590 AA) of peptide exposed outside the membrane, and at least part of this region located in extracellular environment.

Figure 16 shows that the potential proteinase activity for activation of EGF related factors, based on the discussion about the role of rhomboid protein function in the insect

models. The histidine and serine protease activity is able to cleavage intramembranely the transmembrane domain of EGF like factors, resulting release of these ligands to activate the corresponding pathways.

Figure 17 shows a plasmid construct pCI-ICT1024 containing full-length cDNA
5 encoding ICT1024 with CMV promoter driven expression cassette. Transfection of this plasmid into MDA-MD-435 cells resulted in highly expressed ICT1024 protein.

Figure 18 shows a plasmid construct pCI-ICT1024N containing a cDNA fragment of ICT1024 encoding the N-terminal domain. Transfection of this plasmid, which contains a CMV promoter driven expression cassette into MDA-MD-435 cells results in highly
10 expressed ICT1024 protein fragment.

Figure 19 shows the plasmid construct pGEX-5X-3-ICT1024 we built containing full-length cDNA of ICT1024 encoding ICT1024 protein. Transfection of this plasmid with prokaryotic promoter driven expression cassette into bacteria cells resulted in highly expressed ICT1024 protein.

Figure 20 shows the plasmid construct pGEX-5X-3-1024N we built containing cDNA
15 fragment of ICT1024 encoding ICT1024 protein N-terminal domain. Transfection of this plasmid with prokaryotic promoter driven expression cassette into bacteria cells resulted in highly expressed ICT1024 protein fragment.

Figure 21 shows the plasmid construct pGEX-5X-3-ICT1024C we built containing
20 cDNA fragment of ICT1024 encoding ICT1024 protein C-terminal domain. Transfection of this plasmid with prokaryotic promoter driven expression cassette into bacteria cells resulted in highly expressed ICT1024 protein fragment.

Figure 22 shows the plasmid construct pETBlue-2-ICT1024 we built containing full-length cDNA of ICT1024 encoding ICT1024 protein. Transfection of this plasmid with
25 prokaryotic promoter driven expression cassette into bacteria cells resulted in highly expressed ICT1024 protein.

Figure 23 shows the plasmid construct pETBlue-2-ICT1024N containing cDNA fragment of ICT1024 encoding ICT1024 protein N-terminal domain. Transfection of this plasmid with prokaryotic promoter driven expression cassette into bacteria cells resulted in
30 highly expressed ICT1024 protein fragment.

Figure 24 shows the plasmid construct we built containing cDNA fragment of ICT1024 encoding ICT1024 protein C-terminal domain. Transfection of this plasmid with

prokaryotic promoter driven expression cassette into bacteria cells resulted in highly expressed ICT1024 protein fragment.

Figure 25 is the confirmed sequence of ICT1024 protein coding region 1670-3637 (SEQ ID NO:58).

5 Figure 26 (SEQ ID NO:60) is the sequence of the N TERMINUS 553 AA CODING REGION: 1070-2731 of ICT1024

Figure 27, (SEQ ID NO:61) is the sequence of the ICT1024 coding region: 947-3518

Figure 28, (SEQ ID NO:62) is the sequence of the ICT1024 N terminus 553 aa coding region:, 947-2600

10 Figure 29, (SEQ ID NO:64) is the sequence of the ICT1024 coding region for the C terminus 375 aa:, 945-2069

Figure 30, (SEQ ID NO:66) is the sequence of the ICT1024 coding region, 310-2879

Figure 31, (SEQ ID NO:68) is the sequence of the coding region for the N terminus 400 aa of ICT1024, 314-1515

15 Figure 32, (SEQ ID NO:69) Coding region for the C terminus 373 aa of ICT1024: 308-1431

Figure 33 shows the sequence of ICT1025 cDNA, Genebank Accession No. NM_003299, Tumor Rejection Antigen 1 or gp96. (SEQ ID NO:70)

20 Figure 34 shows the sequence of ICT1025 peptide, NP_003290 (SEQ ID NO:71), named as Tumor Rejection Antigen (gp96) 1, Glucose Regulated Protein, grp94 and Endothelial cell glycoprotein.

Figure 35 shows that the ICT1025 siRNA Design: two 21 nt sequences from ICT1025 were selected as the targets for RNAi-mediated knockdown of ICT1025 gene expression. (SEQ ID NO:72 and 73)

25 Figure 36 shows that ICT1025 specific siRNA duplexes are able to knockdown TRA-1 expression in MDA-MB-435 cells, at both message RNA level detected with RT-PCR and protein level detected with Western blot analysis. The knockdown of ICT1025 gene expression with siRNA has demonstrated dose dependent effects.

30 Figure 37 shows that ICT1025 specific siRNA duplexes induce apoptosis activity of MDA-MB-435 cells observed at 48 hours after the transfection.

Figure 38 shows that ICT1025 specific siRNA duplexes decrease cell proliferation of HT-29 cells observed 48 hours after the transfection.

Figure 39 shows that ICT1025 specific siRNA duplexes induce apoptosis activity of HT-29 cells observed at 48 hours after the transfection.

Figure 40 shows that ICT1025 specific siRNA duplexes inhibit growth of MDA-MB-435 cell formed xenograft tumor on nude mice using repeated delivery of the siRNA
5 duplexes. The inhibition of tumor growth caused by ICT1025 knockdown is much stronger than that of hVEGF knockdown.

Figure 41 shows that when the commercial monoclonal antibody specific to ICT1025 was applied on the MDA-MB-435 cells the apoptosis activity of the cells increased dramatically in a dose dependent manner.

10 Figure 42 shows that ICT1025 is located in the membrane fraction of the cell lysates from both MDA-MB-435 cells and MCF-7/VEGF165 cells, detected by the monoclonal antibody.

Figure 43 shows that not only the ICT1025 is located in the membrane fraction, but also presented the extracellular domains on the cell surface, detected by the monoclonal
15 antibody binding of the biotinylated surface proteins.

Figure 44 shows that upregulated expression of ICT1025 in multiple cancer tissues illustrated with a virtual Northern analysis using SEGE database published by NCI.

Figure 45 shows the domain architecture of ICT1025 with a Head Shock Protein 90 domain and a human ATPase_c domain.

20 Figure 46 shows that peptide sequence homology between human TRA-1 and mouse TRA-1 (SEQ ID NO: 71 and 74). The two proteins are highly similar.

Figure 47 shows that the peptide sequence homology between TRA-1 and head shock protein 90 (SEQ ID NO:71 and 75).

Figure 48 shows the predictions of the transmembrane structure of ICT1025.

25 Figure 49 shows the prokaryotic expression vector PGEX53X1025 carrying full-length sequence of ICT1025.

Figure 50 shows the purified ICT1025 protein expressed from the prokaryotic system.

Figure 51 shows the eukaryotic expression vector pCI-ICT1025 carrying the full-length cDNA of ICT1025.

30 Figure 52 shows HLA peptide motif search results.

Figure 53 shows suggested models for transmembrane biology of ICT 1025.

Figure 54 shows predicted transmembrane segments of ICT 1025.

Figure 55 shows screening of ICT 1025 mAB for surface binding activities in breast tumor cells.

Figure 56 shows screening of ICT1025 mAB for surface binding activities in colon tumor cells.

- 5 Figure 57 shows inhibition of tumorigenesis and tumor growth by treating tumor cells with antibody or siRNA prior to inoculation.

DESCRIPTION OF THE INVENTION

The present invention provides validated targets for inhibition of tumor growth,
10 disease progression and methods and compositions for the inhibition and treatment of tumors and cancers, for example, breast cancer, colon cancer, prostate cancer, skin cancer, bone cancer, parotid gland cancer, pancreatic cancer, kidney cancer, uterine cervix cancer, lymph node cancer, or ovarian cancer, in mammals, for example, humans. The invention is based on the findings of novel targets, such as ICT1024, ICT1025, ICT1030, ICT1031, and ICT1003.
15 ICT1030 and/or ICT1031 and/or ICT1003 and/or ICT1024 and/or ICT1025 can thus be used as targets for therapy; and, they also can be used to identify compounds useful in the diagnosis, prevention, and therapy of tumors and cancers (for example, breast cancer, colon cancer, prostate cancer, skin cancer, bone cancer, parotid gland cancer, pancreatic cancer, kidney cancer, uterine cervix cancer, lymph node cancer, ovarian cancer, or lung cancer.

20 The targets ICT1024, ICT1025, ICT1030, ICT1031, and ICT1003, as disclosed herein, are validated by a method of validating drug targets that determines the targets control tumor progression and thus justify anti-tumor drug discovery (see, US Provisional Application No. 60/326,422 and WO03/063765, incorporated herein by reference). This unique and proprietary Tumor Target Discrimination Method validates targets directly in animal tumor
25 models through transgene over-expression and eliminates targets lacking disease control. The method reduces the need for protein generation, antibodies, and/or transgenic animals – all costly and slow, while providing clear and definitive evidence that targets actually control the disease. Moreover, the method provides valuable information that may be lost with methods that rely solely on cell-culture and miss the complex interactions of multiple cell
30 types that result in disease pathology.

The platform technology (see, International Application No. WO 0147496 incorporated by reference), as described above, is a powerful tool for validation of genes that are under-expressed in tumor tissue. However, a technology platform to achieve gene

silencing is highly desired for validation of genes that are over-expressed in tumor tissue. Recently, double stranded RNA has been demonstrated to induce gene specific silencing by a phenomenon called RNA interference (RNAi). Although the mechanism of RNAi is still not completely understood, overwhelming early results suggested that this RNAi effect may be achieved in various cell types including mammalian species. A double stranded RNA targeted against mRNA results in the degradation of the target mRNA causing the silencing of the corresponding gene. Large double stranded RNA is cleaved into smaller fragments, for example, fragments of 21-23 nucleotides long, by a RNase III like activity involving an enzyme Dicer. These shorter fragments known as siRNA (small interfering RNA) are believed to mediate the cleavage of mRNA. The RNAi mechanism for down regulation of gene expression has been studied in *C. elegans* and other lower organisms, its effectiveness in mammalian cells has been demonstrated. Recently, the RNAi effect is demonstrated in mouse using the firefly luciferase gene reporter system (Worby et al. (2001) *Sci STKE* Aug 14, 2001(95):PL1).

Our unique PolyTran™ technology (see, International Application No. WO 0147496) enables direct administration of plasmids into tumor. This provides strong tumor expression and activity of candidate target proteins in the tumor.

Definitions

In general, a "gene" is a region on the genome that is capable of being transcribed to an RNA that either has a regulatory function, a catalytic function, and/or encodes a protein. An eukaryotic gene typically has introns and exons, which may organize to produce different RNA splice variants that encode alternative versions of a mature protein. The skilled artisan will appreciate that the present invention encompasses all endogenous gene that may be found, including splice variants, allelic variants and transcripts that occur because of alternative promoter sites or alternative poly-adenylation sites. The endogenous gene, as described herein, also can be a mutated endogenous gene, and that the mutation can be in the coding or regulatory regions.

A "target gene" refers to a differentially expressed gene in which modulation of the level of gene expression or of gene product activity prevents and/or ameliorates disease progression, for example, a tumor growth. Thus, compounds that modulate the expression of a target gene, the target genes, or the activity of a target gene product can be used in the diagnosis, treatment or prevention of a disease. In particular, target genes in the present invention includes endogenous genes and their variants, as described herein.

A full-length gene or RNA therefore encompasses any naturally occurring splice variants, allelic variants, other alternative transcripts, splice variants generated by recombinant technologies which bear the same function as the naturally occurring variants, and the resulting RNA molecules. A fragment of a gene can be any portion from the gene, which may or may not represent a functional domain, for example, a catalytic domain, a DNA binding domain, etc. A fragment may preferably include nucleotide sequences that encode for at least 16 contiguous amino acids, more preferably at least 25 contiguous amino acids, and most preferably at least about 30, 40, 50, 60, 65, 70, 75 or more contiguous amino acids or any integer thereabout or therebetween. A structural gene is a DNA sequence that is transcribed into messenger RNA (mRNA) which is then translated into a sequence of amino acids characteristic of a specific polypeptide.

"Complementary DNA" (cDNA), often referred to as "copy DNA", is a single-stranded DNA molecule that is formed from an mRNA template by the enzyme reverse transcriptase. Typically, a primer complementary to portions of the mRNA is employed for the initiation of reverse transcription. Those skilled in the art also use the term "cDNA" to refer to a double-stranded DNA molecule that comprises such a single-stranded DNA molecule and its complement DNA strand.

"Gene expression" refers to the biosynthesis of a gene product. For example, in the case of a structural gene, gene expression involves transcription of the structural gene into mRNA and the translation of mRNA into one or more polypeptides.

The term "operably associated" is used to describe the connection between regulatory elements and a gene or its coding region. That is, gene expression is typically placed under the control of certain regulatory elements, including constitutive or inducible promoters, tissue-specific regulatory elements, and enhancers. Such a gene or coding region is the to be "operably linked to" or "operatively linked to" or "operably associated with" the regulatory elements, meaning that the gene or coding region is controlled or influenced by the regulatory element.

"Sequence homology" is used to describe the sequence relationships between two or more nucleic acids, polynucleotides, proteins, or polypeptides, and is understood in the context of and in conjunction with the terms including: (a) reference sequence, (b) comparison window, (c) sequence identity, (d) percentage of sequence identity, and (e) substantial identity or "homologous."

(a) A "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset of or the entirety of a specified sequence; for example, a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence. For polypeptides, the length of the reference polypeptide sequence will generally be at least about 16 amino acids, preferably at least about 20 amino acids, more preferably at least about 25 amino acids, and even more preferably about 35 amino acids, about 50 amino acids, or about 100 amino acids. For nucleic acids, the length of the reference nucleic acid sequence will generally be at least about 50 nucleotides, preferably at least about 60 nucleotides, more preferably at least about 75 nucleotides, and even more preferably about 100 nucleotides or about 300 nucleotides or any integer thereabout or therebetween.

(b) A "comparison window" includes reference to a contiguous and specified segment of a polynucleotide sequence, wherein the polynucleotide sequence may be compared to a reference sequence and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions, substitutions, or deletions (i.e., gaps) compared to the reference sequence (which does not comprise additions, substitutions, or deletions) for optimal alignment of the two sequences. Generally, the comparison window is at least 20 contiguous nucleotides in length, and optionally can be 30, 40, 50, 100, or longer. Those of skill in the art understand that to avoid a misleadingly high similarity to a reference sequence due to inclusion of gaps in the polynucleotide sequence a gap penalty is typically introduced and is subtracted from the number of matches.

Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman, *Adv. Appl. Math.*, 2: 482, 1981; by the homology alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.*, 48: 443, 1970; by the search for similarity method of Pearson and Lipman, *Proc. Natl. Acad. Sci. USA*, 8: 2444, 1988; by computerized implementations of these algorithms, including, but not limited to: CLUSTAL in the PC/Gene program by Intelligenetics, Mountain View, California, GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 7 Science Dr., Madison, Wisconsin, USA; the CLUSTAL program is well described by Higgins and Sharp, *Gene*, 73: 237-244, 1988; Corpet, et al., *Nucleic Acids Research*, 16:881-90, 1988; Huang, et al., *Computer Applications in the Biosciences*, 8:1-6, 1992; and Pearson, et al., *Methods in Molecular Biology*, 24:7-331, 1994. The BLAST family of programs which can be used for database similarity searches includes:

BLASTN for nucleotide query sequences against nucleotide database sequences; BLASTX for nucleotide query sequences against protein database sequences; BLASTP for protein query sequences against protein database sequences; TBLASTN for protein query sequences against nucleotide database sequences; and TBLASTX for nucleotide query sequences
5 against nucleotide database sequences. See, Current Protocols in Molecular Biology, Chapter 19, Ausubel, et al., Eds., Greene Publishing and Wiley-Interscience, New York, 1995. New versions of the above programs or new programs altogether will undoubtedly become available in the future, and can be used with the present invention.

Unless otherwise stated, sequence identity/similarity values provided herein refer to
10 the value obtained using the BLAST 2.0 suite of programs, or their successors, using default parameters. Altschul et al., *Nucleic Acids Res*, 2:3389-3402, 1997. It is to be understood that default settings of these parameters can be readily changed as needed in the future.

As those ordinary skilled in the art will understand, BLAST searches assume that proteins can be modeled as random sequences. However, many real proteins comprise
15 regions of nonrandom sequences which may be homopolymeric tracts, short-period repeats, or regions enriched in one or more amino acids. Such low-complexity regions may be aligned between unrelated proteins even though other regions of the protein are entirely dissimilar. A number of low-complexity filter programs can be employed to reduce such low-complexity alignments. For example, the SEG (Wooten and Federhen, *Comput. Chem.*,
20 17:149-163, 1993) and XNU (Claverie and States, *Comput. Chem.*, 17:191-1, 1993) low-complexity filters can be employed alone or in combination.

(c) "Sequence identity" or "identity" in the context of two nucleic acid or polypeptide sequences includes reference to the residues in the two sequences which are the same when aligned for maximum correspondence over a specified comparison window, and can take into
25 consideration additions, deletions and substitutions. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (for example, charge or hydrophobicity) and therefore do not deleteriously change the functional properties
30 of the molecule. Where sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences which differ by such conservative substitutions are said to have sequence similarity. Approaches for making this adjustment are well-known to those of skill in the art.

Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, for example, according to the algorithm of Meyers and Miller, *Computer Applic. Biol. Sci.*, 4: 11-17, 1988, for example, as implemented in the program PC/GENE (Intelligenetics, Mountain View, California, USA).

(d) "Percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions, substitutions, or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions, substitutions, or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

(e) (i) The term "substantial identity" or "homologous" in their various grammatical forms means that a polynucleotide comprises a sequence that has a desired identity, for example, at least 60% identity, preferably at least 70% sequence identity, more preferably at least 80%, still more preferably at least 90% and even more preferably at least 95%, 96%, 97%, 98%, 99% or 100% identity, compared to a reference sequence using one of the alignment programs described using standard parameters. One of skill will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like. Substantial identity of amino acid sequences for these purposes normally means sequence identity of at least 60%, more preferably at least 70%, 80%, 90%, and even more preferably at least 95%, 96%, 97%, 98%, 99% or 100%.

Another indication that nucleotide sequences are substantially identical is if two molecules hybridize to each other under stringent conditions. However, nucleic acids which do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This may occur, for example,

when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. One indication that two nucleic acid sequences are substantially identical is that the polypeptide which the first nucleic acid encodes is immunologically cross reactive with the polypeptide encoded by the second nucleic acid, although such cross-reactivity is not required for two polypeptides to be deemed substantially identical.

(e) (ii) The terms "substantial identity" or "homologous" in their various grammatical forms in the context of a peptide indicates that a peptide comprises a sequence that has a desired identity, for example, at least 60% identity, preferably at least 70% sequence identity to a reference sequence, more preferably 80%, still more preferably 85%, even more preferably at least 90% or 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the reference sequence over a specified comparison window. Preferably, optimal alignment is conducted using the homology alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.*, 48:443, 1970. An indication that two peptide sequences are substantially identical is that one peptide is immunologically reactive with antibodies raised against the second peptide, although such cross-reactivity is not required for two polypeptides to be deemed substantially identical. Thus, a peptide is substantially identical to a second peptide, for example, where the two peptides differ only by a conservative substitution. Peptides which are "substantially similar" share sequences as noted above except that residue positions which are not identical may differ by conservative amino acid changes. Conservative substitutions typically include, but are not limited to, substitutions within the following groups: glycine and alanine; valine, isoleucine, and leucine; aspartic acid and glutamic acid; asparagine and glutamine; serine and threonine; lysine and arginine; and phenylalanine and tyrosine, and others as known to the skilled person.

The term "antisense RNA" refers to in eukaryotes, RNA polymerase catalyzes the transcription of a structural gene to produce mRNA. A DNA molecule can be designed to contain an RNA polymerase template in which the RNA transcript has a sequence that is complementary to that of a preferred mRNA. The RNA transcript is termed an "antisense RNA." Antisense RNA molecules can inhibit mRNA expression (for example, Rylova et al., *Cancer Res*, 62(3):801-8, 2002; Shim et al., *Int. J. Cancer*, 94(1):6-15, 2001).

The term "antisense DNA" or "DNA decoy" or "decoy molecule" means with respect to a first nucleic acid molecule, a second DNA molecule or a second chimeric nucleic acid molecule that is created with a sequence, which is a complementary sequence or homologous to the complementary sequence of the first molecule or portions thereof, is referred to as the

antisense DNA or DNA decoy or decoy molecule of the first molecule. The term "decoy molecule" also includes a nucleic molecule, which may be single or double stranded, that comprises DNA or PNA (peptide nucleic acid) (Mischiati et al., *Int. J. Mol. Med.*, 9(6):633-9, 2002), and that contains a sequence of a protein binding site, preferably a binding site for a regulatory protein and more preferably a binding site for a transcription factor. Applications of antisense nucleic acid molecules, including antisense DNA and decoy DNA molecules are known in the art, for example, Morishita et al., *Ann. N Y Acad. Sci.*, 947:294-301, 2001; Andratschke et al., *Anticancer Res*, 21:(5)3541-3550, 2001.

"siRNA" refers to small interfering RNAs, which also include short hairpin RNA ("shRNA") (Paddison et al., *Genes & Dev.* 16: 948-958, 2002), that are capable of causing interference (as described herein for RNAi) and can cause post-transcriptional silencing of specific genes in cells, for example, mammalian cells (including human cells) and in the body, for example, mammalian bodies (including humans). The phenomenon of RNA interference (RNAi) is described and discussed in Bass, *Nature*, 411:428-29, 2001; Elbashir et al., *Nature*, 411:494-98, 2001; and Fire et al., *Nature*, 391:806-11, 1998, wherein methods of making interfering RNA also are discussed. Exemplary siRNAs according to the invention could have up to 29 bps, 25 bps, 22 bps, 21 bps, 20 bps, 15 bps, 10 bps, 5 bps or any integer thereabout or therebetween.

A "stabilized RNAi", "siRNA" or a "shRNA" as described herein, is protected against degradation by exonucleases, including RNase, for example, using a nucleotide analogue that is modified at the 3' position of the ribose sugar (for example, by including a substituted or unsubstituted alkyl, alkoxy, alkenyl, alkenyloxy, alkynyl or alkynyloxy group as defined above). The RNAi, siRNA or a shRNA also can be stabilized against degradation at the 3' end by exonucleases by including a 3'-3'-linked dinucleotide structure (Ortigao et al., *Antisense Research and Development* 2:129-146 (1992)) and/or two modified phospho bonds, such as two phosphorothioate bonds.

The RNA molecules used in the electroporation method, as described herein, can be stabilized RNAs. The RNAs of the instant invention include isolated RNAi, siRNA, mRNA or antisense RNA molecule and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) polypeptide or a portion thereof which is capable of binding a target gene motif. A peptide derived from GAPDH is known to bind to and stabilize RNA and may be useful in the stabilization of therapeutic siRNA/RNAi/shRNA molecules in a cell.

The term "cancer" refers to the presence of cells possessing characteristics typical of cancer-causing cells, for example, uncontrolled proliferation, loss of specialized functions, immortality, significant metastatic potential, significant increase in anti-apoptotic activity, rapid growth and proliferation rate, and certain characteristic morphology and cellular markers. In some circumstances, cancer cells will be in the form of a tumor; such cells may exist locally within an animal, or circulate in the blood stream as independent cells, for example, leukemic cells.

The term "tumor" refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all precancerous and cancerous cells and tissues.

The term "precancerous" refers to cells or tissues having characteristics relating to changes that may lead to malignancy or cancer. Examples include adenomatous growths in tissues, or conditions, for example, dysplastic nevus syndrome, a precursor to malignant melanoma of the skin. Examples also include, abnormal neoplastic, in addition to dysplastic nevus syndromes, polyposis syndromes, prostatic dysplasia, and other such neoplasms, whether the precancerous lesions are clinically identifiable or not.

The term "complexed DNA" include a DNA molecule complexed or combined with another molecule, for example, a carbohydrate, for example, a sugar, that a sugar-DNA complex is formed. Such complex, for example, a sugar complexed DNA can enhance or support efficient gene delivery via receptor, for example, glucose can be complexed with DNA and delivered to a cell via receptor, such as mannose receptor.

"Encapsulated nucleic acids", including encapsulated DNA or encapsulated RNA, refer to nucleic acid molecules in microsphere or microparticle and coated with materials that are relatively non-immunogenic and subject to selective enzymatic degradation, for example, synthesized microspheres or microparticles by the complex coacervation of materials, for example, gelatin and chondroitin sulfate (see, for example, US Patent No. 6,410,517). Encapsulated nucleic acids in a microsphere or a microparticle are encapsulated in such a way that it retains its ability to induce expression of its coding sequence (see, for example, US Patent No. 6,406,719).

"Inhibitors" refers to molecules that inhibit and/or block an identified function. Any molecule having potential to inhibit and/or block an identified function can be a "test molecule," as described herein. For example, referring to oncogenic function or anti-apoptotic activity of ICT1024, ICT1025, ICT1031 or ICT1003, such molecules may be identified using in vitro and in vivo assays of ICT1024, ICT1025, ICT1031 or ICT1003,

respectively. Inhibitors are compounds that partially or totally block ICT1024, ICT1025 and/or ICT1031 and/or ICT1003 activity, decrease, prevent, or delay their activation, or desensitize its cellular response. This may be accomplished by binding to ICT1024, ICT1025, ICT1031, or ICT1003 proteins directly or via other intermediate molecules. An antagonist or
5 an antibody that blocks ICT1024, ICT1025 and/or ICT1031 and/or ICT1003 activity, including inhibition of oncogenic function or anti-apoptotic activity of ICT1024, ICT1025, ICT1031 and/or ICT1003, is considered to be such an inhibitor. Inhibitors according to the instant invention is: a siRNA, an RNAi, a shRNA, an antisense RNA, an antisense DNA, a decoy molecule, a decoy DNA, a double stranded DNA, a single-stranded DNA, a complexed
10 DNA, an encapsulated DNA, a viral DNA, a plasmid DNA, a naked RNA, an encapsulated RNA, a viral RNA, a double stranded RNA, a molecule capable of generating RNA interference, or combinations thereof. The group of inhibitors of this invention also includes genetically modified versions of ICT1024, ICT1025, ICT1030, ICT1031, or ICT1003, for example, versions with altered activity. The group thus is inclusive of the naturally occurring
15 protein as well as synthetic ligands, antagonists, agonists, antibodies, small chemical molecules and the like.

"Assays for inhibitors" refer to experimental procedures including, for example, expressing ICT1024, ICT1025, ICT1031, or ICT1003 in vitro, in cells, applying putative inhibitor compounds, and then determining the functional effects on ICT1024, ICT1025,
20 ICT1031, or ICT1003 activity or transcription, as described above. Samples that contain or are suspected of containing ICT1024, ICT1025, ICT1031, or ICT1003 are treated with a potential inhibitor. The extent of inhibition or change is examined by comparing the activity measurement from the samples of interest to control samples. A threshold level is established to assess inhibition. For example, inhibition of a ICT1024, ICT1025, ICT1031, or ICT1003
25 polypeptide is considered achieved when the ICT1024, ICT1025, ICT1031, or ICT1003 activity value relative to the control is 80% or lower.

ICT1030: The term "ICT1030" refers to validated target ICT1030, which includes MFGE8 (Accession No. NM_005928, BC003610), related molecules or consensus, nucleic acid (DNA and RNA) or protein (or polypeptide), and can include their polymorphic variants,
30 alleles, mutants, and interspecies homologs that have (i) substantial nucleotide sequence homology (for example, at least 60% identity, preferably at least 70% sequence identity, more preferably at least 80%, still more preferably at least 90% and even more preferably at least 95%) with the nucleotide sequence of the GenBank Accession No. NM_005928

(protein ID. NP_005919.1), Homo sapiens milk fat globule-EGF factor 8 protein (MFGE8) (protein ID. NP_005919.1); or (ii) at least 65% sequence homology with the amino acid sequence of the GenBank protein_id NP_005919.1 (ICT1030); or (iii) substantial nucleotide sequence homology (for example, at least 60% identity, preferably at least 70% sequence identity to a reference sequence, more preferably 80%, still more preferably 85%, even more preferably at least 90% or 95%) with the nucleotide sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3; or (iv) substantial sequence homology with the encoded amino acid sequence (for example, SEQ ID NO:2).

ICT1030 polynucleotides or polypeptides are typically from a mammal including, but not limited to, human, rat, mouse, hamster, cow, pig, horse, sheep, or any mammal. A "ICT1030 polynucleotide" and a "ICT1030 polypeptide," may be either naturally occurring, recombinant, or synthetic (for example, via chemical synthesis).

MFGE8 DNA sequence contains 1934 base pairs (see SEQ ID NO:1), ICT1030 coding sequence contains 1164 base pairs (see SEQ ID NO:3), encoding a protein of 387 amino acids (see SEQ ID NO:2).

According to an aspect of the present invention, it has been determined that the target ICT1030, for example, MFGE8, is a novel target, a tumor suppressor, in mammalian tissues, including breast tissue, colon tissue, prostate tissue, skin tissue, bone tissue, parotid gland tissue, pancreatic tissue, kidney tissue, uterine cervix tissue, lymph node tissue, and ovarian tissue. Human chromosome region 15q25 is one of the novel targets identified that is validated as a tumor suppressor. Therefore, the tumor-suppressing gene(s) located on chromosome region 15q25 can play an important role in the cancer therapy, including breast, colon, prostate, skin, bone, parotid gland, pancreatic, kidney, uterine cervix, lymph node, and ovarian cancers.

ICT1031: The term "ICT1031" refers to validated target ICT1031, which includes TNFSF13 (Accession Nos. AK090698 and O75888), related molecules such as APRIL, TALL-2 and TRDL-1, or consensus, nucleic acid (DNA and RNA) or protein (or polypeptide), and can include their polymorphic variants, alleles, mutants, and interspecies homologs that have (i) substantial nucleotide sequence homology (for example, at least 60% identity, preferably at least 70% sequence identity, more preferably at least 80%, still more preferably at least 90% and even more preferably at least 95%) with the nucleotide sequence of the Accession No. AK090698, Homo sapiens TNFSF13 (Accession Nos. AK090698 and O75888); or (ii) at least 65% sequence homology with the amino acid sequence of the

Accession No. O75888 (TNFSF13); or (iii) substantial nucleotide sequence homology (for example, at least 60% identity, preferably at least 70% sequence identity to a reference sequence, more preferably 80%, still more preferably 85%, even more preferably at least 90% or 95%) with the nucleotide sequence as set forth in SEQ ID NO:4; or (iv) substantial
5 sequence homology with the encoded amino acid sequence (for example, SEQ ID NO:5).

ICT1031 polynucleotides or polypeptides are typically from a mammal including, but not limited to, human, rat, mouse, hamster, cow, pig, horse, sheep, or any mammal. A "ICT1031 polynucleotide" and a "ICT1031 polypeptide," may be either naturally occurring, recombinant, or synthetic (for example, via chemical synthesis).

10 TNFSF13 DNA sequence (Accession No. AK090698) contains 2036 base pairs (see SEQ ID NO:4) and TNFSF13 encoding protein (Accession No. O75888) contains 250 amino acids (see SEQ ID NO:5).

According to an aspect of the present invention, it has been determined that the target ICT1031, for example, TNFSF13, is a novel target for tumor growth inhibition in mammalian
15 tissues, including breast tissue, colon tissue, esophagus tissue, and ovarian tissue. Therefore, inhibition of tumor-promoting target ICT1031 can play an important role in the cancer therapy, including breast, colon, esophagus, and ovarian cancers.

ICT1003: The term "ICT1003" refers to validated target ICT1003, which includes ZFP236 (Accession Nos. AK000847), related molecules, or consensus, nucleic acid (DNA
20 and RNA) or protein (or polypeptide), and can include their polymorphic variants, alleles, mutants, and interspecies homologs that have (i) substantial nucleotide sequence homology (for example, at least 60% identity, preferably at least 70% sequence identity, more preferably at least 80%, still more preferably at least 90% and even more preferably at least 95%) with the nucleotide sequence of the GenBank Accession No. AK000847, novel Homo
25 sapiens zinc finger protein 236 (GenBank Accession No. AK000847.1); or (ii) at least 65% sequence homology with the amino acid sequence of the protein_id BAA91398.1 (ICTB1003); or (iii) substantial nucleotide sequence homology (for example, at least 60% identity, preferably at least 70% sequence identity to a reference sequence, more preferably 80%, still more preferably 85%, even more preferably at least 90% or 95%) with the
30 nucleotide sequence as set forth in SEQ ID NO:6 or SEQ ID NO:8; or (iv) substantial sequence homology with the encoded amino acid sequence (for example, SEQ ID NO:7).

ICT1003 polynucleotides or polypeptides are typically from a mammal including, but not limited to, human, rat, mouse, hamster, cow, pig, horse, sheep, or any mammal. A

"ICT1003 polynucleotide" and a "ICT1003 polypeptide," may be either naturally occurring, recombinant, or synthetic (for example, via chemical synthesis).

ZFP236 DNA sequence contains 2241 base pairs (see SEQ ID NO:6), ZFP236 coding sequence contains 1419 base pairs (see SEQ ID NO:8), encoding a protein of 472 amino acids (see SEQ ID NO:7).

According to an aspect of the present invention, it has been determined that the target ICT1003, for example, ZFP236, is a novel target for tumor growth inhibition in mammalian tissues, including breast tissue, colon tissue, lung tissue, and ovarian tissue. Therefore, inhibition of tumor-promoting target ICT1003 can play an important role in the cancer therapy, including breast, colon, lung and ovarian cancers.

ICT1024: The term "ICT1024" refers to validated target ICT1024, the gene and protein EGF-AP. The was identified first using a process called Efficacy-First™ discovery (described in PCT/US02/31554, which is hereby incorporated by reference in its entirety). Briefly, human breast tumor carcinoma cells, MDA-MB-435, were inoculated subcutaneously into mouse breast fat pads. When the xenograft tumors grew up to 200mm3 in volume, plasmids expressing basic Fibroblast Growth Factors (FGF-2) were intratumorally delivered repeatedly. The treated tumor demonstrated much faster growth than the untreated tumor.

Tumor tissues was obtained and used to isolate total RNA for microarray analysis (Affymetrix, U133). One of the highly un- or down-regulated genes (about 1% of the total probes on the U133 chip), ICT1024, demonstrated significant up-regulated expression with signal from 585 (control group expression level), to 1208 (treated group expression level). This gene was therefore selected for the next level of target validation with a method called Disease-Control™ validation, using an siRNA based in vivo knockdown in the same xenograft tumor model. Two siRNA duplexes, 21 base pair each (Fig. 7) (SEQ ID NO: 25 and 26), were designed targeting this ICT1024 gene, specific to the sequence of AK026010, NM_022450 and M99624, in the coding region (aagctggacattccctctgcg, aagagcccagcttcctgcgc). Then the two siRNA duplexes were delivered intratumorally three times. The siRNA-mediated knockdown of ICT1024 gene expression resulted in tumor growth inhibition (Fig. 8). The further analysis in a cell culture based study demonstrated that knocking down ICT1024 gene expression in the tumor cell MDA-MB-435, induced a remarkable increase of the apoptosis activity (Fig. 9). Based on these results, ICT1024 was selected for further evaluation as a therapeutic target.

After this identification and validation of the biological function of this gene in cell culture and in a xenograft tumor model, a series of analyses of ICT1024 gene expression profile searches were conducted through both public domain databases and the GeneLogic Express Analysis. The results from the analyses further demonstrated the biological function and its relevance to the disease status, particularly in the area of tumor growth, tumor cell apoptosis and tumor metastasis.

ICT1024 gene is highly expressed in various tumor samples. In addition to the observation from the Efficacy-First™ study that ICT1024 was up-regulated in bFGF treated xenograft tumor formed by inoculation of human breast carcinoma cell, MDA-MB-435 (there is some evidences that this cell line may be from melanoma rather than breast carcinoma), it was found out that ICT1024 is significantly up-regulated in tumor tissue, especially in breast carcinoma tissue samples, from three different analyses. Those analyses were provide online from SAGE Genie databases of NCBI's CANCER GENOME ANATOMY PROJECT. The first analysis is from a SAGE digital Northern (see Table I) (SEQ ID NO:36) that demonstrated a very clearly up-regulated ICT1024 expression in metastatic breast carcinoma tissues. From the same analysis, the up-regulation of ICT1024 gene expression was also observed in stomach cancer, prostate adenocarcinoma, brain glioblastoma and other tumor types. The second analysis is from Monochromatic SAGE/cDNA Virtual Northern (see Table II). In terms of all tissue types, the ICT1024 has been identified as highly up-regulated genes from both EST data set and SAGE data set. In the mammary gland tumor tissue, this gene was significantly up regulated in the SAGE dataset. Brain and prostate are other tissue types showed significant up regulated gene expression in tumor tissues than in the normal tissues. The third analysis is from Two Dimensional Array Display (see Fig. 10). ICT1024 expressions is correlated with the expressions of a group of tumorigenic genes in the breast tumor tissues.

Using Gene Logic's GeneExpress analysis, we found out that not only was ICT1024 up-regulated in the breast tumor tissues, but also was much more up-regulated in the tissues from the stage I tumors than those from other stages of the tumors (Fig. 11). This finding indicates that ICT1024 is actively involved in the early stage of tumor growth.

Through the literature search, ICT1024 was found to have a positive correlation with expression of PSMB1, Proteosome Beta Subunit 1. Proteosome is a multicatalytic proteinase complex and it is able to cleave peptides in a ubiquitin-dependent process. The Ubiquitin-mediated degradation of critical regulators is currently a well-recognized anticancer target

Another positive correlation with RAP1 expression was also been observed. RAP1 is Ras-associated protein-1 and is involved in activation of the Ras oncogene.

ICT1025: The term "ICT1025" refers to validated target ICT1025. The inventors identified the gene and protein, TRA-1, first using a process called Efficacy-First™ discovery (described in PCT/US02/31554, which is hereby incorporated by reference in its entirety).

Briefly, human breast tumor carcinoma cells, MDA-MB-435, were inoculated subcutaneously into mouse breast fat pads. When the xenograft tumors grew up to 200mm³ in volume, plasmids expressing basic Fibroblast Growth Factors (FGF-2) were intratumorally delivered repeatedly. The treated tumor demonstrated much faster growth than the untreated tumor.

Tumor tissues was obtained and used to isolate total RNA for microarray analysis (Affymetrix, U133). One of the highly un- or down-regulated genes (about 1% of the total probes on the U133 chip), ICT1025, demonstrated significant up-regulated expression with signal from 279 (control group expression level), to 412 (treated group expression level).

This gene (Accession No. AK025852, NM_003299 and BC009195, Figure 33, mRNA sequence (SEQ ID NO:70); Figure 34 protein sequence (SEQ ID NO:71) was therefore selected for the next level of target validation with a method called Disease-Control™ validation, using an siRNA based in vivo knockdown in the same xenograft tumor model. Two siRNA duplexes, 21 base pair each (Fig. 35) (SEQ ID NO. 72 and 73), were designed targeting this ICT1025 gene, specific to the sequence in the coding regions of aactgttgaggagcccatgga (started at nt 966) and aatctgatgatgaagctgcag (started at nt 1008). Then the two siRNA duplexes were delivered intratumorally three times. The siRNA-mediated knockdown of ICT1025 gene expression resulted in tumor growth inhibition (Fig. 36). The further analysis in a cell culture based study demonstrated that knocking down ICT1025 gene expression in the tumor cells MDA-MB-435, HT29 induced remarkable increases of the apoptosis activity (Fig. 37) and decrease of cell proliferation. Based on these results, ICT1025 was selected for further evaluation as a therapeutic target.

After this identification and validation of the biological function of this gene in cell culture and in a xenograft tumor model, a series of ICT1025 gene expression profile searches were conducted through both public domain databases and the GeneLogic Express Analysis. The results from the analyses further demonstrated the biological function and its relevance to the disease status, particularly in the areas of tumor growth, tumor cell apoptosis and tumor metastasis..

TRA-1s downregulation by siRNA delivery specifically inhibiting its expression has demonstrated that its expression has a "Disease-Controlling" role in proliferative diseases. By using siRNA-mediated knockdown of TRA-1 expression in the xenograft tumor model it was found that tumor growth rate is inhibited when TRA-1 expression is inhibited. We
5 further found out that knocking down the expression of this protein in several breast tumor cell lines induced significant increase of the apoptotic activity. This finding was further verified when the cells were treated with the monoclonal antibody specifically against this protein. During the process to define the subcellular location of this protein in the breast tumor cells, we found that not only this protein are cell surface membrane bound but also has
10 substantial portion located extracellularly.

Given our findings, one hypothesis for the promising autologous protocol clinical results is that administration of the isolated TRA-1 complex induces antibodies toward TRA-1 itself, not just the autologous patient specific peptides, and contributes substantially to tumor inhibition, or potentially is the major mechanism of activity. Using cell surface
15 biotinylation technique, we observed that existence of TRA-1 proteins in the outer surface of the MDA-MB-435 and MCF-7 breast cancer cells. To explore the biological relevance of the cell surface localization of TRA-1 from breast cancer cells, we examined the mAb on cell apoptosis and proliferation. When the cells were treated with a TRA-1 monoclonal antibody, increase of apoptosis activity and inhibition of cell proliferation were observed. These results
20 strongly suggest an involvement of cell surface TRA-1 in apoptosis and cell proliferation signal pathway. Therefore, TRA-1 inhibitors, including siRNA agent to reduce its expression and antibodies to bind it and inhibit its activity, provide novel and effective treatments for breast cancer, other malignancies, and many proliferative diseases. Success of mAb therapy inhibiting TRA-1 will have broad applications and will be clinical feasible.

25 The invention provides methods and compositions for inhibiting or blocking the biological activity of ICT1025 protein. Therapeutic methods and compositions for treatment of cancer, autoimmune disease and other diseases also are provided. More specifically, the invention provides methods and compositions that permit down-regulation of the production and activity of ICT1025 at the nucleic acid and/or protein level, and that allow deactivation,
30 inhibition, blocking, or down-regulation of biochemical functions of this protein. The inhibition can be achieved, for example, by down-regulating transcription or translation of the protein; by degrading mRNA encoding the protein, by degrading the protein, by blocking and/or deactivating RNA and by inhibiting protein function. The invention provides

inhibitors of ICT1025 including, but not limited to, antibodies, mRNA sequence specific inhibitors, such as siRNA and antisense, peptide antagonists, and small molecule protease inhibitors. The invention also provides methods for generating these inhibitors, and for using one or more inhibitors to achieve a desired biological function, such as treatment and prevention of neoplastic, immunological and/or infectious diseases. In particular, the invention provides immunoglobulin agents, including antibodies and antibody fragments, and methods of using these agents for the treatment and diagnosis of disease. The content of the provisional application Serial No. 60/458,948 is hereby incorporated by reference in its entirety.

10 DNA and protein sequence homology analyses

ICT 1024: There are three full-length cDNA sequences: NM_022450, BC014425 and AK026010, which code for the same protein. The protein contains 855 amino acids and has a molecular weight of about 130 kD.

Using BLAST search for homology of DNA sequences, we found about 325 homologous sequences in human, murine, rattus and fugu, etc. However, only a very few human homologues were found. There is only one cDNA sequence (AK056708) has very high homology with the above three cDNAs, but a short stretch of mutated sequence, which may caused by cloning artifact. Therefore, the protein coding region was disrupted. Other partial cDNA sequences and chromosome sequences were also found. The cDNA was originally named in the NCBI nucleotide database as EGFR related sequence (EGFR-RS), or EGFR related protein (EGFR-RP) before April 22 of 2003. Currently the cDNA has been named as Homo sapiens rhomboid family 1.

We further analyzed the protein sequence of ICT1024. This 855 AA protein has several domain signatures identified by Conserved Domain Architecture Retrieval Tool from NCBI database. One of the major domain structure is a region covering about 146 AA. This domain has been recognized as the conservative region of the Rhomboid family. This family contains integral membrane proteins that are related to Drosophila rhomboid protein. Members of this family are found in bacteria and eukaryotes. Rhomboid promotes the cleavage of the membrane-anchored TGF-alpha-like growth factor Spitz, allowing it to activate the Drosophila EGF receptor (4,5,6,7). Analysis suggests that Rhomboid-1 is a novel intramembrane serine protease that directly cleaves Spitz. These proteins contain three strongly conserved histidines in the putative transmembrane regions that may be involved in the peptidase function. We first compared the ICT1024 rhomboid domain with a group of

rhomboid proteins from various organism (Fig. 12). Although the framework of the ICT1024 rhomboid domain is quite similar to those from other organisms, the sequences are very different. In comparison of the ICT1024 rhomboid domain with other human rhomboid like proteins (Fig. 13), the sequence of ICT1024 is very different from others. In addition, the
5 ICT1024 specific siRNA duplexes used in both in vitro and in vivo validations target regions that are quite different from any of those human rhomboid proteins (Fig. 14).

We also analyzed the hydrophobicity of ICT1024 protein and its potential trans-membrane location. Multiple prediction program have been applied, including SOSUI model (Table III), TMHMM Model and TMPred Models (Fig. 15). From those analyses, it seems
10 that ICT1024 is a integral membrane protein with multiple transmembrane domains and intracellular domains and extracellular domains. Whatever methods used for the predication of the protein location and topology, this protein has been demonstrated with a long N-terminal domain outside the membrane. This N-terminal domain would either be outside of cell or inside the cytoplasm. There are other regions of this protein also exposed to the
15 outside of the cell or cytoplasm. The membrane protein, ICT1024, has a proteinase activity for activation of EGF-EGF receptor pathway and, based on the discoveries described herein, is a very attractive target for therapeutic development of various modalities of drugs, including monoclonal antibody, siRNA inhibitor, peptide antagonist and small molecular inhibitors, etc. A suitable monoclonal antibody will bind to either the extracellular or
20 intracellular domain of the protein and block function of the protein.

ICT 1025

As mentioned in the Background section, there are three full-length cDNA sequences: NM_003299, AK025459 and BC009195, which code for the same protein. The protein
25 contains 803 amino acids.

Using BLAST search for homology of DNA sequences, we found about many homologous sequences in human, murine, rattus and fugu, etc. However, only a very few human homologues were found. There is only one cDNA sequence (AK025459) has very high homology with the above three cDNAs, but a short stretch of mutated sequence, which
30 may caused by cloning art fact. Therefore, the protein coding region was disrupted. Other partial cDNA sequences and chromosome sequences were also found. The cDNA was originally named in the NCBI nucleotide database as Tumor Rejection Protein 1 (TRA-1).

We also analyzed the hydrophobicity of ICT1025 protein and its potential trans-membrane location. Multiple prediction programs including DAS model and TMpred Model were used for the analyses (Fig. 17). Whatever methods used for the predication of the protein location and topology, this protein has been demonstrated with several
5 transmembrane domains. This N-terminal domain would be either outside of cell or inside the cytoplasm. There are other regions of this protein also exposed to the outside of the cell or cytoplasm. The membrane presentation of ICT1025 may play very important roles in tumorigenesis and tumor antigen presenting. Therefore, ICT1025 is a very attractive target for therapeutic development of various modalities of drugs, including monoclonal antibody,
10 siRNA inhibitor, peptide antagonist and small molecular inhibitors, etc. A suitable monoclonal antibody will bind to either the extracellular or intracellular domain of the protein and block function of the protein.

The role in tumor metastasis and growth

15 ICT1024

The protein ICT1024 apparently plays a key role in tumor metastasis and tumor growth, through its activation of the EGF-EGFR pathway, and other proteinase functions and the additional unknown functions. We have evidence to demonstrate that this gene is up-
20 regulated in fast growing tumor from a xenograft tumor model study treated with bFGF expression vector. This gene is up-regulated in mRNA level in tumor tissues, from breast cancer, prostate cancer, brain cancer and other types of cancers, based on the SAGE virtual and digital northern analyses. This gene has also been shown to be up-regulated using Gene Logic's GeneExpress analysis. When the gene expression was knockdown with ICT-1024
25 specific siRNA duplexes in the growing xenograft tumors, the tumor growth was significantly inhibited (Fig. 8).

Apoptosis (programmed cell death) is a form of cellular suicide that typically includes plasma membrane blebbing, cellular volume contraction, and nuclear condensation, and culminates in the activation of endogenous endonucleases that degrade cellular DNA. The
30 well-defined loss of specific cells is crucial during embryonic development and organogenesis. In addition to its physiological roles, apoptosis also occurs in many types of cancer cells when they are exposed to various chemotherapeutic drugs, including antimetabolites, deoxynucleotide synthesis inhibitors, DNA topoisomerase inhibitors, anti-

microtubule agents, alkylating agents, and endoplasmic reticulum (ER) stressors. Interestingly enough, when we knockdown ICT-1024 expression in MDA-MB-435 cells transfected with the specific siRNA duplexes, the apoptosis activity was dramatically increased, as tested with a TUNEL assay, in which terminal deoxynucleotidyl transferase (TdT) catalyzes the incorporation of bromo-deoxyuridine (BrdU) residues into the
5 fragmenting nuclear DNA at the 3'-hydroxyl ends by nicked end labeling.

The specific ICT1024 gene silencing by siRNA duplexes has been verified by RT-PCR. This finding suggested that ICT-1024 plays a crucial role in regulation of tumor cell apoptosis. Other evidence tends to show that the EGF-EGFR is sufficient to activate the
10 major signaling pathways leading to cell proliferation and survival, and EGFR signaling is sufficient to suppress apoptosis induced by serum withdrawal (12).

Malignant tumors grow out of control due to the highly expressed and activated growth factors, EGF, PDGF and VEGF, etc. They penetrate and destroy local tissues and spread throughout the body via blood or the lymphatic system. These tumors are not
15 morphologically typical of the original tissue and are not encapsulated. Malignant tumors commonly recur after surgical removal. Accordingly, treatment ordinarily targets malignant cancers or malignant tumors. The intervention of malignant growth is most effective at the early stage of the cancer development. It is important, therefore, to identify and validate a target for early signs of tumor formation and to determine potent tumor growth or gene
20 expression suppression elements or agents associated therewith. The development of such tumor growth and/or gene expression and therapeutic elements or agents involves an understanding of the genetic control mechanisms for cell division and differentiation, particularly in connection with tumorigenesis.

Based on the GeneExpress analysis, which is based on thousands of clinical samples
25 of tumor tissues and normal tissues, we found that ICT1024 has significantly up-regulated expression in Stage 1 tumor samples (Fig. 11). The signals from Affymetrix array U133 for the Stage 1 tumor samples are much higher (283) than those from the normal tissues (165). All Stage 1 tumor samples showed significant up-regulation of ICT1024 gene expression. Accordingly, ICT1024 is useful as a marker for early cancer diagnosis. It is also very useful
30 for cancer treatment when this gene is specifically knocked down.

In the *Drosophila* cell, the polytopic membrane protein Rhomboid-1 promotes the cleavage of the membrane-anchored TGF α -like growth factor Spitz, allowing it to activate the *Drosophila* EGF receptor. Until now, the mechanism of this key signaling

regulator has remained obscure, but this analysis suggests that Rhomboid-1 is a novel intramembrane serine protease that directly cleaves Spitz. In accordance with the putative Rhomboid active site being in the membrane bilayer, Spitz is cleaved within its transmembrane domain, and thus is the first example of a growth factor activated by regulated intramembrane proteolysis. Rhomboid-1 is conserved throughout evolution from archaea to humans, and these results show that a human Rhomboid promotes Spitz cleavage by a similar mechanism. This growth factor activation mechanism may therefore be widespread (6). Although Rhomboid-1 does not contain any obvious sequence homology domains, it has the characteristics of a serine protease (7). Four of its six essential residues parallel the residues required for a serine protease catalytic triad charge-relay system and an oxyanion stabilization site (consisting of a glycine two residues away from the active serine, and the serine itself; G215 and S217). These are the two active site determinants of serine proteases, and these four essential residues account for all of the amino acids known to participate directly in the serine protease catalytic mechanism (5) These residues are absolutely conserved in all Rhomboids, and their mutation to even very similar residues (i.e., G215A, S217T, and S217C) abolishes Rhomboid-1 activity. These are hallmarks of active site residues. (3) The location of the essential residues is highly suggestive of a serine protease active site; both G215 and S217 occur in the conserved GASGG motif, which is remarkably similar to the conserved GDSGG motif surrounding the active serine of 200 different serine proteases. Furthermore, the essential residues N169 and H281 occur at the same height in their transmembrane domains (TMDs) as the GASGG motif, consistent with the proposal that they associate with S217 to generate a catalytic triad. Finally, Spitz processing is directly inhibited by the specific serine protease inhibitors DCI and TPCK, and Rhomboid-1 itself becomes limiting in their presence, suggesting that Rhomboid-1 is their direct target and thus the serine protease responsible for Spitz cleavage. The suggested model is presented in Figure 16.

Because of our understanding about this gene and its encoded protein, and its potential function in human cell, we designate this gene as EGF Activation Protein (EGF-AP). We also conclude that EGF-AP is an attractive cancer target for anti-tumor therapeutic development. Inhibitors, such as DNA binding protein, RNA binding protein, siRNA or other types of RNAi, antisense, ribozyme and DNAzyme, etc., that are able to block the ICT1024 protein production, are effective for treating diseases associated with increased ICT1024 expression. In addition, these diseases also may be treated using inhibitors, such as

monoclonal antibodies, polyclonal antibodies, single chain antibodies, intrabodies, protein antagonists, small molecule protease inhibitors or other types of inhibitors, will be effective blockers of ICT1024 protein functions. We have also recognized that this ICT1024 protein may represent a novel class of drug targets useful for treatment of cancer and other diseases.

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5 13 Patrick Lu, Frank Xie et al. US Application No. 60/458,948 Targets for Tumor Treatment

ICT1025

The protein ICT1025 apparently plays a key role in tumor metastasis and tumor
10 growth, through its multiple roles as inhibitor of apoptosis, activator of proliferation and up-regulation of multiple drug resistant genes. We have evidence to demonstrate that this gene is up-regulated in fast growing tumor from a xenograft tumor model study treated with bFGF expression vector. This gene is up-regulated in mRNA level in tumor tissues, from breast cancer, prostate cancer, brain cancer and other types of cancers, based on the SAGE virtual
15 and digital northern analyses. This gene has also been shown to be up-regulated using Gene Logic's GeneExpress analysis. When the gene expression was knockdown with ICT1025 specific siRNA duplexes in the growing xenograft tumors, the tumor growth was significantly inhibited (Fig. 38).

Apoptosis (programmed cell death) is a form of cellular suicide that typically includes
20 plasma membrane blebbing, cellular volume contraction, and nuclear condensation, and culminates in the activation of endogenous endonucleases that degrade cellular DNA. The well-defined loss of specific cells is crucial during embryonic development and organogenesis. In addition to its physiological roles, apoptosis also occurs in many types of cancer cells when they are exposed to various chemotherapeutic drugs, including
25 antimetabolites, deoxynucleotide synthesis inhibitors, DNA topoisomerase inhibitors, anti-microtubule agents, alkylating agents, and endoplasmic reticulum (ER) stressors. Interestingly enough, when we knockdown ICT1025 expression in MDA-MB-435 cells and HT-29 cells, transfected with the specific siRNA duplexes, the apoptosis activity was dramatically increased (Figure 37 and 38), as tested with a TUNEL assay, in which terminal
30 deoxynucleotidyl transferase (TdT) catalyzes the incorporation of bromo-deoxyuridine (BrdU) residues into the fragmenting nuclear DNA at the 3'-hydroxyl ends by nicked end labeling.

The specific ICT1025 gene silencing by siRNA duplexes has been verified by RT-PCR (Fig. 36). This finding suggested that ICT1025 plays a crucial role in regulation of

tumor cell apoptosis. Other evidence tends to show that the ICT1025 is sufficient to activate the major signaling pathways leading to cell proliferation and survival.

Methods of Treating Cellular Proliferative Diseases

5 Using inhibitors to block ICT1024 and ICT1025 protein production

RNAi, antisense, ribozyme and other nucleic acid therapeutics can be used to inhibit expression of ICT 1003 and ICT-1024 and ICT 1025 and ICT 1031 in patients suffering diseases with cellular proliferation. For example, an ICT 1003 or ICT-1024 or ICT 1025 or
10 ICT 1031 antisense strand (either RNA or DNA) is directly introduced into the cells in a form that is capable of binding to the mRNA transcripts. Alternatively, a vector containing a sequence which once within the target cells, is transcribed into the appropriate antisense mRNA, may be administered. Antisense nucleic acids which hybridize to target mRNA decrease or inhibit production of the polypeptide product encoded by a gene by associating
15 with the normally single-stranded mRNA transcript, thereby interfering with translation and thus, expression of the protein. For example, DNA containing a promoter, e.g., a tissue-specific or tumor specific promoter, is operably linked to a DNA sequence (an antisense template), which is transcribed into an antisense RNA. By "operably linked" is meant that a coding sequence and a regulatory sequence(s) (i.e., a promoter) are connected in such a way
20 as to permit gene expression when the appropriate molecules (e.g., transcriptional activator proteins) are bound to the regulatory sequence(s).

Oligonucleotides complementary to various portions of ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 can be determined in vitro for their ability to decrease production of ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 in human cells (e.g., using the FOCUS
25 hepatocellular carcinoma (HCC) cell line) according to standard methods. A reduction in ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 gene product in cells contacted with the candidate antisense composition compared to cells cultured in the absence of the candidate composition is detected using ICT 1003 or ICT-1024 or ICT 1025 or ICT1031-specific antibodies or other detection strategies. Sequences which decrease production of ICT 1003 or
30 ICT-1024 or ICT 1025 or ICT1031 in in vitro cell-based or cell-free assays are then be tested in vivo in rats or mice to confirm decreased ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 production in animals with malignant neoplasms.

Antisense therapy is carried out by administering to a patient an antisense nucleic acid by standard vectors and/or gene delivery systems. Suitable gene delivery systems may include liposomes, polymers, receptor-mediated delivery systems, naked DNA, and viral vectors such as herpes viruses, retroviruses, adenoviruses and adeno-associated viruses, among others. A therapeutic nucleic acid composition is formulated in a pharmaceutically acceptable carrier. The therapeutic composition may also include a gene delivery system as described above. Pharmaceutically acceptable carriers are biologically compatible vehicles which are suitable for administration to an animal: e.g., physiological saline. A therapeutically effective amount of a compound is an amount which is capable of producing a medically desirable result such as reduced production of an ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 gene product or a reduction in cellular proliferation in a treated animal.

Parenteral administration, such as intravenous, subcutaneous, intramuscular, and intraperitoneal delivery routes, may be used to deliver nucleic acids or ICT 1003 or ICT-1024 or ICT 1025 or ICT1031-inhibitory peptides on non-peptide compounds. Liposome formulations of therapeutic compounds may also facilitate activity.

Dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular nucleic acid to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Dosage for intravenous administration of nucleic acids is from approximately 10⁶ to 10²² copies of the nucleic acid molecule.

RNA interference (RNAi) is a post-transcriptional process where the double-stranded RNA (dsRNA) inhibits gene expression in a sequence specific fashion. The RNAi process occurs in at least two steps: in first step, the longer dsRNA is cleaved by an endogenous ribonuclease into shorter, less than 100-, 50-, 30-, 23-, or 21-nucleotide-long dsRNAs, termed "small interfering RNAs" or siRNAs. In second step, the smaller siRNAs mediate the degradation of the target mRNA molecule. This RNAi effect can be achieved by introducing either longer dsRNA or shorter siRNA to the target sequence within cells. It is also demonstrated that RNAi effect can be achieved by introducing plasmids that generate dsRNA complementary to target gene. The RNAi have been successfully used in gene function determination in *Drosophila* (Kennerdell et al. (2000) *Nature Biotech* 18: 896-898; Worthy et al. (2001) *Sci STKE* Aug 14, 2001(95):PL1; Schmid et al. (2002) *Trends Neurosci* 25(2):71-74; Hammond et al. (2000). *Nature*, 404: 293-298), *C. elegans* (Tabara et al. (1998) *Science* 282: 430-431; Kamath et al. (2000) *Genome Biology* 2: 2.1-2.10; Grishok et al. (2000)

Science 287: 2494-2497), and Zebrafish (Kennerdell et al. (2000) *Nature Biotech* 18: 896-898). In those model organisms, it has been reported that both the chemically synthesized shorter siRNA or in vitro transcribed longer dsRNA can effectively inhibit target gene expression. There are increasing reports on successfully achieved RNAi effects in non-

5 human mammalian and human cell cultures (Manche et al. (1992). *Mol. Cell. Biol.* 12:5238-5248; Minks et al. (1979). *J. Biol. Chem.* 254:10180-10183; Yang et al. (2001) *Mol. Cell. Biol.* 21(22):7807-7816; Paddison et al. (2002). *Proc. Natl. Acad. Sci. USA* 99(3):1443-1448; Elbashir et al. (2001) *Genes Dev* 15(2):188-200; Elbashir et al. (2001) *Nature* 411: 494-498; Caplen et al. (2001) *Proc. Natl. Acad. Sci. USA* 98: 9746-9747; Holen et al. (2002) *Nucleic*

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15 *USA* 99(6):5515-5520; Yu et al. (2002) *Proc. Natl. Acad. Sci. USA* 99(9):6047-6052). The two siRNA duplexes we have used can effectively silence ICT1024 or EGF-AP expression in both cell based assay and xenograft tumor model. However, there are regions of mRNA of ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 are useful for siRNA targeted knockdown.

In another aspect, the invention provides methods for inhibiting cancer or

20 precancerous growth in a mammalian tissue, comprising contacting the tissue with an inhibitor that interacts with the target ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 DNA or RNA and thereby inhibits the target ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 gene expression, wherein the tissue is breast tissue, colon tissue, prostate tissue, skin tissue, bone tissue, parotid gland tissue, pancreatic tissue, kidney tissue, uterine cervix tissue, lymph node

25 tissue, or ovarian tissue, wherein the inhibitor is an a nucleic acid molecule, a decoy molecule, a decoy DNA, a double stranded DNA, a single-stranded DNA, a complexed DNA, an encapsulated DNA, a viral DNA, a plasmid DNA, a naked RNA, an encapsulated RNA, a viral RNA, a double stranded RNA, a molecule capable of blocking the target ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 gene expression, or combinations thereof.

30 Another aspect of the invention provides methods of administering inhibitors to a patient in need thereof, wherein the inhibitor molecule is delivered in the form of a monoclonal antibody, a peptide antagonist, a small molecule protease blocker, a naked

oligonucleotide or a vector, wherein the nucleic acid interacts with the target ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 gene.

Yet another aspect of the invention provides methods of administering an inhibitor to a patient in need thereof, wherein the inhibitor molecule is delivered in the form of a naked
5 oligonucleotide or a vector, wherein the nucleic acid interacts with the target ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 gene, wherein the nucleic acid is delivered as a vector, wherein the vector is a plasmid, cosmid, bacteriophage, or a virus, for example, a retrovirus or an adenovirus based vector.

Still another aspect of the invention provides methods of blocking in vivo expression
10 of a gene by administering a vector to a patient in need thereof, wherein the vector containing target ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 gene, wherein the nucleic acid interacts with the target ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 gene expression, wherein the nucleic acid inhibits the target ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 gene expression in a mammalian cell, for example, a human cell.

15 According to another aspect of the invention, the inhibitor molecules are introduced into tissues, including breast tissue, colon tissue, prostate tissue, skin tissue, bone tissue, parotid gland tissue, pancreatic tissue, kidney tissue, uterine cervix tissue, lung tissue, lymph node tissue, or ovarian tissue.

20 Using inhibitors to block protein function

Antibody inhibitors of ICT 1003 or ICT-1024 or ICT 1025 or ICT1031

The present invention provides compositions and methods for treatments or
diagnostics for diseases which progress by cellular proliferation, such as proliferation or metastasis of solid tumors, arthritis in rheumatoid arthritis, diabetic retinopathy, retinopathy
25 of prematurity, psoriasis, and the like.

The inventors of the present invention find that antibodies binding to ICT1024 and capable of recognizing an epitope present in a region of the 1st to 590th positions from the N-terminal amino acid can specifically react with the human ICT1024 by immunocyte staining, and that biological activities can be inhibited by the inhibition of binding. Diagnosis and
30 treatment of the above-described diseases in which their morbid states progress by abnormal angiogenesis, such as proliferation or metastasis of solid tumors, arthritis in rheumatoid arthritis, diabetic retinopathy, prematurity retinopathy and psoriasis, can be carried out by using these monoclonal antibodies.

Consequently, the present invention provides antibodies which specifically react with human ICT1024. With regard to the monoclonal antibody of the present invention, a monoclonal antibody is provided that recognizes an epitope which is present in a region of the 1st to 590th, for example, between positions 161-190 or 451-480 as measured from the N-terminal amino acid. A C-terminal epitope region is between positions 740-855, for example positions 826-855. The present invention also provides a monoclonal antibody which inhibits binding of human ICT1024 and also inhibits biological activities of ICT1024.

The inventors of the present invention find that antibodies binding to ICT1025 and capable of recognizing an epitope present in a region of the 1st to 300th positions from the N-terminal amino acid can specifically react with the human ICT1025 by immunocyte staining, and that biological activities can be inhibited by the inhibition of binding. Diagnosis and treatment of the above-described diseases in which their morbid states progress by abnormal angiogenesis, such as proliferation or metastasis of solid tumors, arthritis in rheumatoid arthritis, diabetic retinopathy, prematurity retinopathy and psoriasis, can be carried out by using these monoclonal antibodies.

Consequently, the present invention provides antibodies which specifically react with human ICT1025. With regard to the monoclonal antibody of the present invention, a monoclonal antibody is provided that recognizes an epitope which is present in a region of the 1st to 300th, for example, between positions 161-190 or 251-280 as measured from the N-terminal amino acid. A C-terminal epitope region is between positions 700-803, for example positions 726-803. The present invention also provides a monoclonal antibody which inhibits binding of human ICT1025 and also inhibits biological activities of ICT1025.

The monoclonal antibody of the present invention may be any antibody, so long as it specifically reacts with human ICT 1003 or ICT-1024 or ICT 1025 or ICT1031. Examples of the monoclonal antibody include an antibody produced by a hybridoma and a recombinant antibody produced by a transformant transformed with an expression vector containing the antibody gene. For example, those which are established with murine or rabbit hybridomas can be prepared. That is, anti-human ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 monoclonal antibody can be obtained by preparing human ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 protein as an antigen, immunizing an animal capable of providing a hybridoma with the antigen, such as mouse, rat, hamster, rabbit or the like, thereby inducing plasma cells having the antigen specificity, preparing a hybridoma capable of producing the monoclonal antibody through fusion of the cells with a myeloma cell line and subsequently

culturing the hybridoma. Alternatively, anti-human ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 monoclonal antibody can be obtained by preparing plasmids expressing human ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 protein, immunizing an animal capable of providing a hybridoma with the antigen using DNA vaccination, such as mouse, rat, hamster, rabbit or the like, thereby inducing plasma cells having the antigen specificity, preparing a hybridoma capable of producing the monoclonal antibody through fusion of the cells with a myeloma cell line and subsequently culturing the hybridoma.

Alternatively, a fully human antibody that binds ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 protein can be isolated from a human antibody library using phage display methods, as described in , for example, in United States Patent, 5,885,793, the contents of which are hereby incorporated by reference in their entirety. Human antibodies also can be isolated from transgenic xenomice that have been modified to encode a portion of the human immunoglobulin repertoire, as described for example, in United States Patent 6,075,181, the contents of which are hereby incorporated by reference in their entirety. Alternatively, camelid-type antibodies that lack light chains may be used, as described, for example, in United States Patent 5,800,988 the contents of which are hereby incorporated by reference in their entirety.

The monoclonal antibody which specifically reacts with human ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 of the present invention may be a recombinant antibody. Examples of the recombinant antibody includes a humanized antibody and an antibody fragment. The recombinant antibody of the present invention can be obtained by modifying the above-described monoclonal antibody of the present invention using gene recombination technique. The recombinant antibody includes antibodies produced by gene recombination, such as a humanized antibody and an antibody fragment (e.g., single chain antibody, disulfide stabilized antibody). Among these, antibodies which have the characteristics of monoclonal antibodies, show low antigenicity and have prolonged half-life in blood are preferred as therapeutic agents. The humanized antibody of the present invention includes a human chimeric antibody and a human CDR (complementarity-determining region; hereinafter referred to as "CDR")-grafted antibody. The antibody fragment of the present invention includes a fragment of antigen binding (hereinafter referred to as "Fab"), Fab', F(ab')₂, a single chain antibody (single chain Fv; hereinafter referred to as "scFv"), and a disulfide stabilized antibody (disulfide stabilized Fv; hereinafter referred to as "dsFv"), which specifically react with ICT1024. The antibody also may be a "diabody" of the type described

in U.S. Patent No. 5,837,242, the contents of which are hereby incorporated by reference in their entirety

The antibody which reacts with human ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 of the present invention may be a humanized antibody which is selected from a human chimeric antibody and a human CDR-grafted antibody.

The structure of the antibody of the present invention may belong to any immunoglobulin (Ig) class, but preferably contains the C region of IgG type immunoglobulin, particularly of IgG subclasses, such as IgG1, IgG2, IgG3, and IgG4.

In addition, the present invention relates to the following methods:

a method for immunologically detecting human, comprising reacting human ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 with the antibody or peptide of the present invention;

a method for immunologically detecting cells in which human ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 is expressed on the surface thereof, comprising reacting human ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 with the antibody or peptide of the present invention;

a method for inhibiting binding of human ICT 1003 or ICT-1024 or ICT 1025 or ICT1031, comprising reacting human ICT1024 with the antibody or peptide of the present invention;

a method for inhibiting biological activities of human ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 with the antibody or peptide of the present invention;

a method for detecting a disease in which the morbid states progress by abnormal cell proliferation, comprising reacting a sample with the antibody or peptide of the present invention; and

a method for preventing or treating a disease, comprising the step of administering to human or animal in need of such prevention or treatment an effective amount of the antibody or peptide of the present invention.

In the above method for immunologically detecting human ICT 1003 or ICT-1024 or ICT 1025 or ICT1031, the human ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 or a fragment of ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 may be soluble.

In the above method for inhibiting biological activities of human ICT 1003 or ICT-1024 or ICT 1025 or ICT1031, for example, the activity of human ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 is inhibited.

In the above method for detecting a disease, for example, the method may comprise (a) separating human cell or a crushing solution thereof, tissue or a crushing solution thereof, serum, pleural fluid, ascites fluid, or ocular fluid to prepare a sample, (b) reacting the separated sample prepared in the step (a) with the monoclonal antibody or peptide of the present invention, (c) further reacting the reacted sample prepared in the step (b) with a labeled anti-mouse IgG antibody or binding fragment, and (d) measuring or observing the labeled sample prepared in the step (c).

In the above method for preventing or treating a disease, examples of the disease include diseases in which the morbid states progress by abnormal cellular proliferation. Examples of the diseases in which their morbid states progress by abnormal cellular proliferation include proliferation or metastasis of solid tumor, arthritis in chronic rheumatoid arthritis, diabetic retinopathy, retinopathy of prematurity, and psoriasis. Examples of the solid tumor include breast cancer, prostatic cancer, large bowel cancer, gastric cancer and lung cancer.

The present invention relates to a composition comprising the antibody or peptide of the present invention and a diagnostic or pharmaceutical acceptable carrier.

Patients with tumors characterized as expressing or overexpressing ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 such as tumors are treated by administering ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 antibodies

ICT 1003 or ICT-1024 or ICT 1025 or ICT1031-specific antibodies will inhibit proliferation of cells in culture and in pathological tissues. Different ICT-1024 or ICT1025-specific antibodies can be developed and demonstrated to inhibit cell proliferation. For example, tumor cells (a hepatocarcinoma cell line, a lung carcinoma cell line, and a breast carcinoma cell line) can be seeded in a 96 well plate and incubated with varying concentrations of antibody for 48 hours. The cells can be fixed and cell growth monitored using a sulforhodamine B dye binding assay. The data indicate a reduction in cell viability and proliferation in the presence of ICT 1003 or ICT-1024 or ICT 1025 or ICT1031-specific antibody compared to in its absence.

Passive Immunization

Purified antibody preparations (e.g., a purified monoclonal antibody, an antibody fragment, or single chain antibody) is administered to an individual diagnosed with a tumor

or at risk of developing a tumor. The antibody preparations are administered using methods known in the art of passive immunization, e.g., intravenously or intramuscularly. The antibodies used in the methods described herein are formulated in a physiologically-acceptable excipient. Such excipients, e.g., physiological saline, are known in the art.

5 The antibody is preferably a high-affinity antibody, e.g., an IgG-class antibody or fragment or single chain thereof. Alternatively, the antibody is an IgM isotype. Antibodies are monoclonal, e.g., a murine monoclonal antibody or fragment thereof, or a murine monoclonal antibody, which has been humanized. The antibody is a human monoclonal antibody. The affinity of a given monoclonal antibody is further increased using known
10 methods, e.g., by selecting for increasingly higher binding capacity (e.g., according to the method described in Boder et al., 2000, *Proc. Natl. Acad. Sci. U.S.A.* 97:10701-10705). Optionally, the antibody, antibody fragment, or high affinity single chain antibody is conjugated to a toxic moiety prior to administration. Toxic moieties suitable for conjugation include ricin, Pseudomonas toxin, Diphtheria toxin as well as radioisotopes and
15 chemotherapeutic agents known in the art. Such antibody toxins damage or kill a tumor cell upon binding to the tumor cell or upon internalization into the cytoplasm of the tumor cell.

Antibody preparations or antibody-toxin preparations are administered at doses of approximately 0.01-2 mL/kg of body weight. Doses are readministered daily, weekly, or monthly as necessary to reduce tumor load in a treated individual.

20 Active vaccination is the process of inducing an animal to respond to an antigen. During vaccination, cells, which recognize the antigen (B cells or cytotoxic T cells), are clonally expanded. In addition, the population of helper T cells specific for the antigen also increase. Vaccination also involves specialized antigen presenting cells, which can process the antigen and display it in a form which can stimulate one of the two pathways. Antigen
25 recognition followed by immune cell expansion and activation leads to the production of antigen-specific antibodies and antigen-specific cellular immune responses. Successful immunization is indicated by an increase in the level of ICT 1003 or ICT-1024 or ICT 1025 or ICT1031-specific antibody titer in serum of an immunized individual compared to the level prior to immunization. Preferably, the ICT 1003 or ICT-1024 or ICT 1025 or ICT1031-
30 specific antibody titer is at least 10%, more preferably at least 50%, more preferably at least 100%, and most preferably 200% greater than the titer prior to immunization.

For active immunization, an individual is immunized with an ICT 1003 or ICT-1024 or ICT 1025 or ICT1031 polypeptide or a polynucleotide encoding the peptide. For example,

a human patient is immunized with full-length ICT 1003 or ICT-1024 or ICT 1025 or ICT1031. Standard adjuvant formulations may be simultaneously administered to enhance immunogenicity of the immunizing polypeptide. Alternatively, shorter polypeptides, e.g., immunogenic fragments of ICT 1003 or ICT-1024 or ICT 1025 or ICT1031, are used. For
5 example, a polypeptide contains an extracellular catalytic domain of ICT-1024 (e.g., amino acids 1-590 of ICT-1024. Other immunogenic fragments of ICT-1024 include fragments within the region of amino acids 1-590. A polypeptide containing the extracellular domain of ICT 1025.

Monoclonal antibody therapy is a passive immunotherapy because the antibodies are
10 produced in large quantities outside the body rather than by the immune system of human body itself. This type of therapy can be effective even if the immune system is weakened, which is a typical case for cancer patients. These treatments do not require the immune system to take an "active" role in fighting the cancer. Antibodies are mass-produced by fusing a myeloma cell from a mouse with a mouse B cell that makes a specific antibody. The cell
15 that results from this fusion is called a hybridoma. The combination of a B cell that can recognize a particular antigen and a myeloma cell that lives indefinitely makes the hybridoma cell a kind of perpetual antibody-producing factory. Because the antibodies are all identical clones produced from a single (mono) hybridoma cell, they are called monoclonal antibodies. The monoclonal antibodies that react with specific antigens, e.g. ICT 1003 or ICT-1024 or
20 ICT 1025 or ICT1031, on certain types of cancer cells, are able to neutralize the targeted protein or block its biological function. As a result, the EGF-AP is deactivated and the EGF pathway was shut down and tumor growth is inhibited.

Antibody therapy can be applied in following way: A. Naked Monoclonal Antibodies, the antibodies attach themselves to specific antigens on cancer cells. B. Conjugated
25 monoclonal antibodies are joined to drugs, toxins, or radioactive atoms, and used as delivery vehicles to take those substances directly to the cancer cells. The MAb acts as a homing device, circulating in the body until it is attracted by, and attaches itself to, a cancer cell with a matching antigen. It delivers the toxic substance to where it is needed most, minimizing damage to normal cells in other parts of the body. But conjugated antibodies still generally
30 cause more side effects than do naked antibodies. C. Immunotoxins are made by attaching toxins (poisonous substances from plants or bacteria) to monoclonal antibodies. Various immunotoxins have been made by attaching monoclonal antibodies to bacterial toxins such as diphtherial toxin (DT) or pseudomonal exotoxin (PE40), or to plant toxins such as ricin A or

saporin. D. Until recently, the effectiveness of MAb therapies was limited by the fact that the antibodies were produced by mouse hybridoma cells. In some cases, these antibodies worked well at first. But after a while, the patient's immune system would recognize the mouse antibodies as "foreign" and would destroy them. For this reason, the humanized Mab was
 5 generated by combining the part of the mouse antibody gene responsible for recognizing a specific tumor antigen with other parts from a human antibody gene. The product of this mouse-human antibody gene, looks enough like a normal human antibody to avoid being destroyed by the patient's own immune system. This helps the antibody to be effective for longer periods. All the above stated approaches are useful for ICT1024 or EGF-AP based
 10 antibody therapy. Other approaches, like intrabody, single chain antibody and DNA vaccine can also be used to generate antibody agents for research, diagnosis and therapeutic applications.

In the present invention, two different embodiments for antibody inhibitors according to the transmembrane topology of ICT1024: N-terminal is located outside of cell, vs. N-
 15 terminal is located inside of cell. When the N-terminal is located outside the cell, a large fragment from 1st to 409th AA is preferred to be the antigen for antibody generation, either using the entire 409 AA peptides or different portion within this fragment. There are several sequences may serve as good antigens due to their ligation strength to a defined HLA type: GLSAPHTPV (174TH) (SEQ ID NO: 43), GMQKIIDPL (151TH)(SEQ ID NO: 44),
 20 KMSFRAAAA (213)(SEQ ID NO:45) and LTAEEPSFL (30) (SEQ ID NO:46). Design the antigen peptides containing those sequence will increase the binding activity of the induced antibodies. Only several short peptide fragments will be outside of the cell in this scenario, which may not be strong antigen for generation of antibodies to bind to ICT1024. When the N-terminal is located inside of cell, another long fragment of the ICT1024 protein, from
 25 433th to 660th AA, is presumably located outside the cell. In this case, the fragment provides a good antigen as whole, or multiple antigens selected within the region. There are several strong HLA binding motifs in the region: SQHETVDSV (433TH) (SEQ ID NO:47), GYENVKYYV (446TH)(SEQ ID NO: 48), YVQQENFWI (453TH) (SEQ ID NO:49), and LLPFLNPEV (641TH) (SEQ ID NO:50).

30 There is one scenario that the C-Terminal domain is located outside of cell. The short fragment from 823 to 855 AA can also serve as a peptide antigen either with the entire sequence or partial of the sequence.

Three 30 AA peptides were selected as the examples for generations of polyclonal and monoclonal antibodies:

N'-RGRAFRVADDTAEGLSAPHTPVTPGAASLC-C' (161-190th)(SEQ ID NO:51);

5 N'-VKYVQQENFWIGPSSEALIHLGAKFSPCMR-C' (451-480th) (SEQ ID NO: 52);

N'-PVRCEWCEFLTICIPFTDKFCEKYELDAQLH-C' (826-855th) (SEQ ID NO:53).

10 The similar peptide sequences can also be selected as potential peptide antigens with size from 14 AA to more than 100 AA.

In the present invention, two different embodiments for antibody inhibitors according to the transmembrane topology of ICT1025: N-terminal is located outside of cell, vs. N-terminal is located inside of cell. When the N-terminal is located outside the cell, a large fragment from 1st to 300th AA is preferred to be the antigen for antibody generation, either using the entire 300 AA peptides or different portion within this fragment. There are several sequences may serve as good antigens due to their ligation strength to a defined HLA type: ALWVLGLCC (3TH) (SEQ ID NO:76), VLGLCCVLL (6TH)(SEQ ID NO:77), LLHVTDTGV (144TH)(SEQ ID NO:78) and SELIGQFGV (189TH)(SEQ ID NO:79). Design the antigen peptides containing those sequence will increase the binding activity of the induced antibodies. Only several short peptide fragments will be outside of the cell in this scenario, which may not be strong antigen for generation of antibodies to bind to ICT1025. There is one scenario that the C-Terminal domain is located outside of cell. The short fragment from 823 to 855 AA can also serve as a peptide antigen either with the entire sequence or partial of the sequence.

25 Three 30 AA peptides were selected as the examples for generations of polyclonal and monoclonal antibodies:

N'-ADDEVVDVDGTVEEDLGKSREGSRTDDEVVQ-C' (21-50th) (SEQ ID No: 80);

30 N'-SAFLVADKVIVTSKHNNDTQHIWESDSNEF-C' (201-230th) (SEQ ID No: 81);

N'-SEKTKESREAVEKEFEPLLNWMKDKALKDK-C' (701-730th) (SEQ ID No: 82).

The similar peptide sequences can also be selected as potential peptide antigens with size from 14 AA to more than 100 AA.

According to another aspect of the invention, the inhibitor molecules are introduced into tissues, including breast tissue, colon tissue, prostate tissue, skin tissue, bone tissue,
5 parotid gland tissue, pancreatic tissue, kidney tissue, uterine cervix tissue, lung tissue, lymph node tissue, or ovarian tissue.

Inhibitory protease inhibitors.

Peptide antagonists, small molecule protease inhibitors and other types of ICT1024 inhibitors are also provided to block or inhibit ICT1024 activity.

10 The following examples are offered to illustrate embodiments of the present invention, but should not be viewed as limiting the scope of the invention.

EXAMPLES:

15 Example 1. Gene Delivery Method for Target Validation:

Efficacy-First Discovery™ method is initiated with a known gene that acts as a key player in a defined disease pathway (for example, angiogenesis) and a well-defined disease model (for example, human tumor xenografted in nude mice). An effective gene delivery tool is crucial, that is, one with strong expression but, equally or more importantly, with little
20 background activity from the delivery tool itself. A non-viral and polymer based delivery system can provide both strong delivery and low background for solid tumors. The pathological, pharmaceutical and histological readouts following the treatment are analyzed in comparison with gene expression and protein profiles. Based on both bioinformatics analysis and biological analysis, the genes and proteins significantly up or down regulated in
25 the defined pathway can be carefully selected and further analyzed by the same iterative in vivo validation process. The process started with effective gene delivery into the tumor tissue. The affected tumors were first evaluated by growth rate, histological changes, and then harvested for expression profile analysis with Affymetrix Chips. The highly up- or down-regulated targets were identified for Disease-Control validation. Novel targets were
30 validated in vivo.

The targets identified by Efficacy-First Discovery™ method are different from those identified using a conventional approach. The advantage of Efficacy-First Discovery™

method is that the targets selected by the method is associated with disease efficacy and not simply with a disease state (see Figure 1). The expression changes of the targets are due to perturbation of delivered genes and disease process dynamic. They are better fit for drug discovery.

5 Example 2. Tumor Perturbation with Known Factors.

The human breast cancer carcinoma cell induced xenograft tumors were perturbed with genes that are known to affect tumor growth, and forced to grow faster and slower. The xenograft tumor model was induced with MDA-MB-435 cell on Ncr nu/nu mice. This demonstration was performed using the proprietary polymer mediated IL-2 and bFGF
10 deliveries, based on our previous data and that bFGF is a well-known drug target enhancing tumor growth, and IL-2, which is not only a target but an approved cancer inhibition drug. Four tumor samples treated with IL-2, 4 tumor samples treated with bFGF, and 2 samples treated with Luc as control were collected and processed. When tumor reached 50 mm³ in size, pCI-IL-2 and pCI-bFGF were directly delivered intratumorally with pCI-Luc as a
15 control. Tumor tissues (10 in total) were harvested at different time points and RNA samples were isolated by RNAsol, quantified and gel verified for their integrities. Data show tumor growth inhibition by IL-2 and enhancement by bFGF.

Example 3. Expression Analysis with Affymetrix Chip.

The total RNA samples from tumor tissues were subjected to expression analysis
20 using Affymetrix GeneChip U133 A. The pictures show the original array images. The treated samples were compared with the control samples and initial analytical data were further analyzed with bioinformatics effort. According to the tumor growth rate and efficacy data, combined with the bioinformatics data and literature search, we used at least two folds as a benchmark for significant regulated targets. The signals must be higher than 200.

25 Example 4. Novel Targets Identified.

According to the perturbation effects on the tumor growth, bioinformatics analysis and literature search, only small percentage of gene targets were selected based on their expression profile changes. For example, 156 targets were selected based on about 23,000 pairs of comparisons from Affymetrix U133 A chip. The tumor tissue was harvested 24
30 hours after the second injection of IL-2 expressing vector. Among 156 selected targets, 111 of them were known based on UniGene database annotations, versus 45 were unknown novel

targets. Within the known targets, 87% are tumor related. If the same ratio holds the truth, we expect more than 35 targets are novel tumor targets. In addition, the hits also belong to several tumorigenesis pathways.

Many of these selected targets are well known and some of them are at different stages of clinical development (see Table 1). The long list of unknown targets (no annotation in UniGene Database) holds great potential as novel tumor targets. For further validation in xenograft tumor models, more than 200 targets were selected from both IL-2 and bFGF treated samples (see Table 2). The strategy for the validation is screening those targets with established procedure followed by more comprehensive study of each of the positive hits.

Among 156 selected targets (see Table 1), many of them are well characterized and at different stages of clinical studies. These examples indicate that the targets selected either known or unknown are having great potentials.

Based on expression analyses of 8 tissue samples treated by both IL-2 and bFGF, we selected highly up-or down-regulated targets. About 2/3 of the targets are known and 1/3 are novel according to UniGene database annotations. Selected targets, either known or novel, were subjected to the Disease-Control Validation.

Example 5. Target Validation Process.

Recently disclosed new technology platform, using RNAi mediated in vivo gene silencing for validating drug targets controlling tumor disease (see U. S. provisional application serial no. 60/401,029), was used. This invention further validates the technology platform by performing a complete set of experiments studying payloads and delivery methods on tumor-bearing mouse models.

1) Target Validation: Tumor Correlation or Control

Of the many levels of drug target validation, the ultimate is demonstration that a candidate target actually controls the disease. Disease controlling targets are the high value targets that justify drug discovery. The goal of drug development is products that selectively target key pathways and the key controlling elements of those pathways in order to provide effective therapeutic control of the disease. Validation of such key pathways and elements requires demonstration that addition or subtraction of individual candidate targets controls the disease, i.e. results in a clear increase or decrease of pathology. In vitro cell-based strategies have provided useful information in helping identify and select potential targets. However,

the ability of targets to control in vitro cell models associated with disease frequently is not sufficient to prove the target actually controls the disease process, i.e. the complex interactions of multiple cell types that result in disease pathology. Definitive demonstration of disease control by targets can only be obtained by studies of those targets in a true disease model.

The process of target discovery has been greatly accelerated by genomic methods but validation remains a bottleneck. First-generation genomic methods have generated large pools of candidate targets piled up at the validation step. Many approaches are currently being used to study the function of these gene targets and to validate their role in a disease process. Many of these approaches, although having the benefit of being efficient and high throughput, often succeed only at establishing a correlation or association with disease processes rather than determining a controlling role. Newer gene knockdown and forward or inverse genomic approaches have proven useful but these identify genes whose inhibition or mutation may have a disease role, missing potential valuable information from a gene's over-expression. Furthermore, they also employ primarily in vitro cell-based phenotypes, which do not reflect the complex multi-cellular mechanisms of most diseases, such as tumor angiogenesis, and hence run the risk of missing important targets in adjacent cellular pathways or provide disease associations which are incomplete without the full biological context.

2) Rapid Definitive Target Validation

We have recently disclosed two technology platforms for validating cancer-related drug targets that addresses many of these limitations and has a valuable complementary role in the target validation process. Both unique and proprietary Target Discrimination Methods validate targets directly in animal tumor models by over-expressing transgene(s) or silencing endogenous gene(s) in tumor tissue. The methods reduce the need for the costly and slow steps of definitive validation, such as gene cloning and sequencing, generation of proteins and antibodies or transgenic animals. The combination of these two methods vastly accelerates the process, and most importantly rapidly eliminates weaker targets. Moreover, results obtained by the methods provide clear and definitive evidence that targets actually control the disease, the key validation needed to proceed to the costly steps of drug discovery. The methods can be used to complete the validation of any candidate targets such as those generated from cell culture, model organisms, transgenic animals, etc.

3) Target Discovery: Capturing Targets Missed in Preliminary Validation

Another critical consideration is that, unfortunately, many high value disease-controlling targets may be lost when in vitro or disease-association methods are employed as the first “filter” in target discovery and validation. Many disease-controller targets may only
5 be found in the context of the entire disease model. For example, targets controlling angiogenesis of tumors will only be found at the conjunction of tumors and blood vessels. In the case of tumors, certain valuable targets may only be discovered by studying the in vivo biological system containing assembly of tumor and surrounding tissues.

4) High throughput Target Discovery Solutions

10 We have also disclosed a method for discovering disease controller targets. The method is to scale-up the basic approach so that it can be applied to screen larger sets of gene targets in a higher throughput operation. By scaling the method to processing 1000 candidate genes in animal tumor models each quarter with our in vivo gene delivery technology, this approach can provide the opportunity to skip or shorten, in many cases, preliminary
15 functional validation methods.

5) Tumor Target Elimination

The disclosed technologies also permit candidate targets to be rapidly tested for their capacity in controlling tumor growth. Those candidates showing only weak or negligible control of tumor growth can be eliminated from consideration in favor of those that have a
20 strong effect on tumor growth. These Tumor Target Discrimination Methods rapidly discriminates targets into three categories: those enhancing tumor growth, those with little effect on tumor growth, and those inhibiting tumor growth.

Example 6. Novel Targets Validated.

Both known and unknown targets (see example 4) were selected for Disease-Control
25 Validation in the tumor models. Based on proprietary nucleic acid delivery technologies, two different platforms are established for understanding disease-control property of each target, by either knockdown or over-express the expression. Using a highly efficient method for siRNA delivery in vivo, several groups of targets have been validated. The novel tumorigenesis related targets are identified and validated (see Table 3). On the other hand,
30 we also applied over-expression approach to validate a group of known angiogenesis related gene targets in the same xenograft tumor model with a proprietary delivery. IL-12 were

clearly re-validated based on their roles in tumor growth inhibition. The same approach is currently used for novel target validation also.

Table 3. siRNA-mediated validation of several groups of targets.

Two siRNA targets were selected for each gene and verified by BLAST, and
5 synthesized by Dharmacon (Lafayette, CO). A 10 μ g of specific siRNA for each gene was repeatedly delivered intratumorally into MDA-MB-435 xenograft model. Tumor sizes were measured with N = 8 or N=10. A. Group I targets were validated using xenografted tumor model. Human VEGF and mouse VEGFR2 were used as the positive controls. Three targets were validated among the Group I targets (see Table 3, Group I). B. Group II targets were
10 also validated with the same type assay. Two targets were validated among the Group II (see Table 3, Group II).

The Group III validation included some of previously validated targets and some novel targets. One novel target was validated in the Group III.

When the following targets, ICT1024, ICT1025, ICT1031, ICT1030, and ICT1003,
15 were down regulated by two duplexes of specific siRNA molecules, the tumor growth rates changed. Among them, ICT1030, milk fat globule-EGF factor 8 protein or breast epithelial BA46 antigen, GeneBank Accession Nos.: NM_005928, BC003610 and their splicing derivatives, behaved more like a tumor suppressor target, or a protein therapy and gene therapy target. Since the siRNA-mediated knockdown resulted tumor growth acceleration
20 rather than inhibition. Other targets: ICT1031 (GeneBank no.: AK090698, Tumor Necrosis Factor ligand super family member 13, or TNF related proliferation inducing ligand and their splicing variants, see Figure 3), and ICT1003 (GeneBank no.: AK000847, human novel zinc finger protein 236 or its splicing variants), are all up regulated in fast growing tumor, and have demonstrated as the suitable targets for antibody, small molecules, antisense, siRNA
25 and other antagonist agents.

Among the selected targets tested with siRNA knockdown in vivo, 4 targets (ICT1024, ICT1025, ICT1030 and ICT1031) were validated with n=8 and n=10 (8 and 10 tumors per cohort, respectively) (see Figures 3-6). Two proteins are cell surface factors with totally opposite effects. ICT1030 knockdown by specific siRNA resulted in tumor grow
30 enhancement, versus ICT1031 knockdown triggered tumor growth inhibition. Therefore, the former may be protein or gene therapy drug and the later can be an antibody or a small molecular drug target.

One of the selected targets, ICT1003, was tested with siRNA knockdown in vivo (8 tumors per cohort). The target ICT1003 is a novel zinc finger protein and may represent a transcription factor. ICT1003 knockdown by specific siRNA resulted in tumor growth inhibition (see Figure 4). Therefore, the protein can be a siRNA drug target or a small molecular drug target.

Example 7: Small Interfering RNA (siRNA):

Sense and antisense siRNAs duplexes are made based upon targeted region of a DNA sequence for targets ICT1024, ICT1025, ICT1030, ICT1031, or ICT1003, as disclosed herein (see, for example, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or a fragment thereof), are typically less than 100 base pairs ("bps") in length and constituency and preferably are about 30 bps or shorter, and are made by approaches known in the art, including the use of complementary DNA strands or synthetic approaches. SiRNA derivatives employing polynucleic acid modification techniques, such as peptide nucleic acids, also can be employed according to the invention. The siRNAs are capable of causing interference and can cause post-transcriptional silencing of specific genes in cells, for example, mammalian cells (including human cells) and in the body, for example, mammalian bodies (including humans). Exemplary siRNAs according to the invention have up to 29 bps, 25 bps, 22 bps, 21 bps, 20 bps, 15 bps, 10 bps, 5 bps or any integer thereabout or therebetween.

A targeted region is selected from the DNA sequence (for example, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or a fragment thereof). Various strategies are followed in selecting target regions and designing siRNA oligos, for example, 5' or 3' UTRs and regions nearby the start codon should be avoided, as these may be richer in regulatory protein binding sites. Designed sequences preferably include AA-(N21 or less nucleotides)-TT and with about 30% to 70% G/C-content. If no suitable sequences are found, the fragment size is extended to sequences AA(N29 nucleotides). The sequence of the sense siRNA corresponds to, for example, (N21 nucleotides)-TT or N29 nucleotides, respectively. In the latter case, the 3' end of the sense siRNA is converted to TT. The rationale for this sequence conversion is to generate a symmetric duplex with respect to the sequence composition of the sense and antisense 3' overhangs. It is believed that symmetric 3' overhangs help to ensure that the small interfering ribonucleoprotein particles (siRNPs) are formed with approximately equal ratios of sense and antisense target RNA-cleaving siRNPs (Elbashir et al. *Genes & Dev.* 15:188-200, 2001).

ICT1024 siRNA: Sense or antisense siRNAs are designed based upon targeted regions of a DNA sequence, as disclosed herein (see SEQ ID NO:3), and include fragments having up to 29 bps, 25 bps, 22 bps, 21 bps, 20 bps, 15 bps, 10 bps, 5 bps or any integer thereabout or therebetween. For example, 21 bps siRNA targeting the sense strand of mRNA include:

5'-AAGCTGGACATTCCCTCTGCG-3' (SEQ ID NO:21) and

5'-AAGAGCCCAGCTTCCTGCAGC-3' (SEQ ID NO:22).

ICT1025 siRNA: Sense or antisense siRNAs are designed based upon targeted regions of a DNA sequence, as disclosed herein (see SEQ ID NO:3), and include fragments having up to 29 bps, 25 bps, 22 bps, 21 bps, 20 bps, 15 bps, 10 bps, 5 bps or any integer thereabout or therebetween. For example, 21 bps siRNA targeting the sense strand of mRNA include:

5'-AACTGTTGAGGAGCCCATGGA-3' (SEQ ID NO:23) and

5'-AATCTGATGATGAAGCTGCAG-3' (SEQ ID NO:24)

ICT1030 siRNA: Sense or antisense siRNAs are designed based upon targeted regions of a DNA sequence, as disclosed herein (see SEQ ID NO:3), and include fragments having up to 29 bps, 25 bps, 22 bps, 21 bps, 20 bps, 15 bps, 10 bps, 5 bps or any integer thereabout or therebetween. For example, 21 bps siRNA include:

Targeted region (base position numbers 88-108, (SEQ ID NO:9) 5'-aaccctgccacaacggtggt-3', and the corresponding sense siRNA (SEQ ID NO:10), 5'-aaccctgccacaacggtggt-3';

Targeted region (base position numbers 190-210, SEQ ID NO:11) 5'-aaccactgtgagacgaaatgt-3', and the corresponding sense siRNA (SEQ ID NO:12) 5'-aaccacUgUgagacgaaaUgU-3'; and continuing in this progression to the end of ICTE1030 coding sequence, as set forth herein.

A set of siRNAs/shRNAs are designed based on ICT1030-coding sequence (SEQ ID NO:3).

ICT1031 siRNA: Sense or antisense siRNAs are designed based upon targeted regions of a DNA sequence, as disclosed herein (see SEQ ID NO:4), and include fragments having up to 29 bps, 25 bps, 22 bps, 21 bps, 20 bps, 15 bps, 10 bps, 5 bps or any integer thereabout or therebetween. For example, 21 bps siRNA include:

Targeted region (base position numbers 90-110, (SEQ ID NO:13)

5'-aactgccccagcgatctctgc-3', and the corresponding sense siRNA (SEQ ID NO:14), 5'-aacUgccccagcgaUcUcUgc-3';

Targeted region (base position numbers 330-310, (SEQ ID NO:15)

5'-aacctaattctcctgaggctg-3', and the corresponding sense siRNA (SEQ ID NO:16) 5'-aaccUaaUUcUccUgaggcUg-3'; and continuing in this progression to the end of ICTB1031 coding sequence, as set forth herein.

A set of siRNAs/shRNAs are designed based on ICT1031-coding sequence (SEQ ID NO:4).

ICT1003 siRNA: Sense or antisense siRNAs are designed based upon targeted regions of a DNA sequence, as disclosed herein (see SEQ ID NO:6), and include fragments having up to 29 bps, 25 bps, 22 bps, 21 bps, 20 bps, 15 bps, 10 bps, 5 bps or any integer thereabout or therebetween. For example, 21 bps siRNA include:

Targeted region (base position numbers 345-365, (SEQ ID NO:17) 5'-aatgcggagaacactaattat-3', and the corresponding sense siRNA (SEQ ID NO:18), 5'-aaUgcggagaacacUaaUUaU-3';

Targeted region (base position numbers 462-482, (SEQ ID NO:19) 5'-aatgacaagccacatcgatgt-3', and the corresponding sense siRNA (SEQ ID NO:20) 5'-aatgacaagccacatcgatgt-3'; and continuing in this progression to the end of ICTB1003 coding sequence, as set forth herein.

A set of siRNAs/shRNAs are designed based on ICTB1003-coding sequence (see SEQ ID NO:6).

Experimental Details for Development of ICT1024 Antibodies

Generation of expression vectors for ICT1024 protein or peptide

1.1 Plasmid DNA based mammalian gene expression vectors consist of a eukaryotic gene promoter or a viral gene promoter, a multiple cloning site sequence, and a polyA signal sequence. The promoters include, but not limit to, CMV promoter, RSV promoter, SV40 promoter, EF promoter, E2F promoter, and E1 gene promoter of adenovirus. The polyA sequences include, but not limit to, bGH polyA, SV40 polyA, and synthetic polyA.

1.2 Viral vector based mammalian gene expression vectors include, but not limit to, retroviral vectors, adenoviral vectors, and baculoviral vectors.

1.3 Bacterial expression vectors include, but not limit to, pQE-based vectors, pGEX-based vectors, and pETBlue vector.

1.4 Yeast expression vectors include but not limit to, pESC vectors, p42K-TEF, and pFastBac.

1.5 Cytoplasmic expression vectors utilizing prokaryotic promoters that include, but not limit to, T7 promoter, sp6 promoter.

5 Example 8 Cloning of ICT1024 full-length cDNA into pCI vector for mammalian cell expression and DNA vaccination

The full-length cDNA of ICT1024 (855 aa) was generated by PCR amplification using a cDNA clone purchased from ATCC (MGC: 20194) as template. Since the full-length of ICT1024 cDNA is 2568 bp, to reduce the mutation may occur during the PCR reaction,
10 two pairs of primers were designed to generate two shorter DNA fragments that can be ligated together to generate full-length ICT1024 cDNA.

Primer 1: 1024EcoUp2 (28-mer, corresponds to 111-128 nt of ICT1024 gene, GenBank Accession Number: BC014425) (SEQ ID NO: 54)

5'---C AGG AAT TCC ATG AGT GAG GCC CGC AGG---3'

15 Primer 2: 1024MidDn (26-mer, corresponds to 1770-1745 nt of ICT1024 gene, GenBank Accession Number: BC014425)(SEQ ID NO:55)

5'---CC CTG GGA TCC TGG TGG CAG ACA GAG---3'

Primer 3: 1024SalDn (29-mer, corresponds to 2678-2661 nt of ICT1024 gene, GenBank Accession Number: BC014425) (SEQ ID NO: 56)

20 5'---CC GGC GTC GAC TCA GTG GAG CTG AGC GTC---3'

Primer 4: 1024MidUp (26-mer, corresponds to 1755-1780 nt of ICT1024 gene, GenBank Accession Number: BC014425) (SEQ ID NO:57)

5'--- CA CCA GGA TCC CAG GGT GTG TGA TGA---3'

PCR reaction using Primer 1/ Primer 2 and MGC 20194 template generated a 1679 bp
25 DNA fragment that contains the 111 to 1770 nt of the ICT1024. PCR reaction using Primer 3/ Primer 4 and MGC 20194 template generated a 928 bp DNA fragment that contains the 1755 to 2678 nt of the ICT1024. After purification of the PCR products, the 1679 bp DNA fragment was digested with EcoRI and BamHI, the 928 bp fragment was digested with BamHI and Sal I, then cloned into pCI vector cleaved with EcoRI and Sal I through a three-
30 fragment ligation reaction. The final product, pCI-ICT1024 plasmid DNA, was identified and its sequence was confirmed by DNA sequencing.

See Fig. 17. for restriction map of pCI-ICT1024 expression plasmid. See Fig. 25 for sequence of ICT1024 protein coding region 1670-3637 (SEQ ID NO 58).

Example 9. Cloning of the cDNA fragment coding the N terminus peptide (553 aa) of ICT1024 into pCI vector for mammalian cell expression and DNA vaccination

The cDNA coding for the N terminus 553aa of ICT1024 was generated by a PCR amplification using a MGC20194 DNA as template. One pair of primers was used to generate
 5 the 1679 bp cDNA fragment containing a EcoRI site at its 5' and a Sal I site at its 3' end. Also, a TGA termination codon was integrated into the end of coding region to ensure correct stop of translation.

Primer 1: 1024EcoUp2 (28-mer, corresponds to 111-128 nt of ICT1024 gene,
 GenBank Accession Number: BC014425) (SEQ ID NO:54)

10 5'---C AGG AAT TCC ATG AGT GAG GCC CGC AGG---3'

Primer 5: 1024MDnSal (29-mer, with SalI site+TGA+1769-1755 nt of ICT1024 gene,
 GenBank Accession Number: BC014425) (SEQ ID NO: 59)

5'---CC CTG GTCGAC TCA cct ggg atc ctg gtg---3'

PCR reaction using Primer 1/ Primer 5 and MGC 20194 template generated a 1679 bp
 15 DNA fragment that contains the 111 to 1769 nt of the ICT1024. After purification of the PCR products, the 1679 bp DNA fragment was digested with EcoRI and Sal I, and then cloned into pCI vector cleaved with EcoRI and Sal I. The final product, pCI-ICT1024N plasmid DNA, was identified and its sequence was confirmed by DNA sequencing.

See Fig. 18 for the restriction map of pCI-ICT1024N plasmid. See Fig. 26 for the
 20 sequence of ICT1024 N Terminus 553 amino acid coding region nt. 1070-2731 (SEQ ID NO: 60)

Example 10. Cloning of ICT1024 full-length cDNA into pGEX-5X-3 vector for protein expression in E.coli host

The full-length cDNA of ICT1024 (855 aa) was generated by PCR amplification
 25 using a cDNA clone purchased from ATCC (MGC: 20194) as template. Since the full-length of ICT1024 cDNA is 2568 bp, to reduce the mutation may occur during the PCR reaction, two pairs of primers were designed to generate two shorter DNA fragments that can be ligated together to generate full-length ICT1024 cDNA.

Primer 1: 1024EcoUp2 (28-mer, corresponds to 111-128 nt of ICT1024 gene,
 30 GenBank Accession Number: BC014425) (SEQ ID NO:54)

5'---C AGG AAT TCC ATG AGT GAG GCC CGC AGG---3'

Primer 2: 1024MidDn (26-mer, corresponds to 1770-1745 nt of ICT1024 gene,
 GenBank Accession Number: BC014425) (SEQ ID NO:55)

5'---CC CTG GGA TCC TGG TGG CAG ACA GAG---3'

Primer 3: 1024SalDn (29-mer, corresponds to 2678-2661 nt of ICT1024 gene,

GenBank Accession Number: BC014425) (SEQ ID NO:56)

5'---CC GGC GTC GAC TCA GTG GAG CTG AGC GTC---3'

5 Primer 4: 1024MidUp (26-mer, corresponds to 1755-1780 nt of ICT1024 gene,

GenBank Accession Number: BC014425) (SEQ ID NO:57)

5'--- CA CCA GGA TCC CAG GGT GTG TGA TGA---3'

PCR reaction using Primer 1/ Primer 2 and MGC 20194 template generated a 1679 bp DNA fragment that contains the 111 to 1770 nt of the ICT1024. PCR reaction using Primer 3/ Primer 4 and MGC 20194 template generated a 928 bp DNA fragment that contains the 1755 to 2678 nt of the ICT1024. After purification of the PCR products, the 1679 bp DNA fragment was digested with EcoRI and BamHI, the 928 bp fragment was digested with BamHI and Sal I, then cloned into pGEX-5X-3 vector (Amersham) cleaved with EcoRI and Sal I through a three-fragment ligation reaction. The pGEX-5X-3 is a bacterial expression vector utilizes the bacterial tac promoter to drive the expression of a GST domain (27 Kd) fusion protein. The final product, pGEX-5X-3-ICT1024 plasmid DNA, was identified and its sequence was confirmed by DNA sequencing.

See Fig. 27 for confirmed sequence of pGEX-5X-3-ICT1024 (SEQ ID NO:61)

Example 11. Cloning of the cDNA fragment coding the N terminus peptide (553 aa) of ICT1024 into pGEX-5X-3 vector for protein expression in E.coli

The cDNA coding for the N terminus 553aa of ICT1024 was generated by a PCR amplification using a MGC20194 DNA as template. One pair of primers was used to generate the 1679 bp cDNA fragment containing a EcoRI site at its 5' and a Sal I site at its 3' end. Also, a TGA termination codon was integrated into the end of coding region to ensure correct stop of translation.

Primer 1: 1024EcoUp2 (28-mer, corresponds to 111-128 nt of ICT1024 gene, GenBank Accession Number: BC014425) (SEQ ID NO:54)

5'---C AGG AAT TCC ATG AGT GAG GCC CGC AGG---3'

Primer 5: 1024MDnSal (29-mer, with SalI site+TGA+1769-1755 nt of ICT1024 gene, GenBank Accession Number: BC014425) (SEQ ID NO:59)

5'---CC CTG GTCGAC TCA cct ggg atc ctg gtg---3'

PCR reaction using Primer 1/ Primer 5 and MGC 20194 template generated a 1679 bp DNA fragment that contains the 111 to 1769 nt of the ICT1024. After purification of the

PCR products, the 1679 bp DNA fragment was digested with EcoRI and Sal I, and then cloned into pGEX-5X-3 vector cleaved with EcoRI and Sal I. The final product, pGEX-5X-3-ICT1024N plasmid DNA, was identified and its sequence was confirmed by DNA sequencing.

- 5 See Fig. 14 for restriction map of pGEX-5X-3-1024N. See Fig. 28 for sequence (SEQ ID NO:62)

Example 12. Cloning of the cDNA fragment coding the C terminus peptide (372 aa) of ICT1024 into pGEX-5X-3 vector for protein expression in E.coli

- 10 The cDNA coding for the C-terminal 372 aa of ICT1024 was generated by a PCR amplification using a MGC20194 DNA as template. One pair of primers was used to generate the 1141 bp cDNA fragment containing a EcoRI site at its 5' and a Sal I site at its 3' end.

Primer 6: 1024midEcoUp (30-mer, with EcoRI site 1560-1577 nt of ICT1024 gene, GenBank Accession Number: BC014425) (SEQ ID NO:63)

5'--- CCC AGG AAT TCC CAG GTG CAC AGC TTC ATT---3'

- 15 Primer 3: 1024SalDn (29-mer, corresponds to 2678-2661 nt of ICT1024 gene, GenBank Accession Number: BC014425) (SEQ ID NO:56)

5'---CC GGC GTC GAC TCA GTG GAG CTG AGC GTC---3'

- 20 PCR reaction using Primer 6/ Primer 3 and MGC 20194 template generated a 1141 bp DNA fragment that contains the 1560 to 2678 nt of the ICT1024. After purification of the PCR products, the 1141 bp DNA fragment was digested with EcoRI and Sal I, and then cloned into pGEX-5X-3 vector cleaved with EcoRI and Sal I. The final product, pGEX-5X-3-ICT1024C plasmid DNA, was identified and its sequence was confirmed by DNA sequencing.

- 25 See Fig. 15 for confirmed sequence of pGEX-5X-3-ICT1024C. See Fig. 27 for the sequence ((SEQ ID NO:64)

Example 13. Cloning of ICT1024 full-length cDNA into pETBlue-2 vector for protein expression in E.coli host

- 30 The full-length cDNA of ICT1024 (855 aa) was generated by PCR amplification using a cDNA clone purchased from ATCC (MGC: 20194) as template. Since the full-length of ICT1024 cDNA is 2568 bp, to reduce the mutation may occur during the PCR reaction, two pairs of primers were designed to generate two shorter DNA fragments that can be ligated together to generate full-length ICT1024 cDNA.

Primer 1: 1024EcoUp2 (28-mer, corresponds to 111-128 nt of ICT1024 gene,
GenBank Accession Number: BC014425) (SEQ ID NO:54)

5'---C AGG AAT TCC ATG AGT GAG GCC CGC AGG---3'

Primer 2: 1024MidDn (26-mer, corresponds to 1770-1745 nt of ICT1024 gene,
GenBank Accession Number: BC014425) (SEQ ID NO:55)

5'---CC CTG GGA TCC TGG TGG CAG ACA GAG---3'

Primer 3: 1024SalDn (29-mer, corresponds to 2678-2661 nt of ICT1024 gene,
GenBank Accession Number: BC014425) (SEQ ID NO:56)

5'---CC GGC GTC GAC TCA GTG GAG CTG AGC GTC---3'

Primer 8: 1024ClaDn (30-mer, corresponds to 2675-2658 nt of ICT-1024, GenBank
Accession Number: BC014425) (SEQ ID NO:65)

5'---CGC GGC ATC GAT GTG GAG CTG AGC GTC CAG---3'

PCR reaction using Primer 1/ Primer 2 and MGC 20194 template generated a 1679 bp
DNA fragment that contains the 111 to 1770 nt of the ICT1024. PCR reaction using Primer
3/ Primer 8 and MGC 20194 template generated a 925 bp DNA fragment that contains the
1755 to 2675 nt of the ICT1024. After purification of the PCR products, the 1679 bp DNA
fragment was digested with EcoRI and BamHI, the 928 bp fragment was digested with
BamHI and Cla I, then cloned into pETBlue-2 vector (Novagen) cleaved with EcoRI and Cla
I through a three-fragment ligation reaction. The final product, pETBlue-2-ICT1024 plasmid
DNA, was identified and its sequence was confirmed by DNA sequencing.

See Fig. 28 for restriction map of pETBlue-2-ICT1024 plasmid. See Fig. 30 for
sequence. (SEQ ID NO:66)

Example 14. Cloning of the cDNA fragment coding the N terminus peptide (400 aa) of
ICT1024 into pETBlue-2 vector for protein expression in E.coli

The cDNA coding for the N terminus 400 aa of ICT1024 was generated by a PCR
amplification using a MGC20194 DNA as template. One pair of primers was used to generate
the 1221 bp cDNA fragment containing a EcoRI site at its 5' and a Cla I site at its 3' end. No
stop codon was integrated into the end of coding region since there is a stop codon
downstream of the pETBlue-2 vector.

Primer 1: 1024EcoUp2 (28-mer, corresponds to 111-128 nt of ICT1024 gene,
GenBank Accession Number: BC014425) (SEQ ID NO:54)

5'---C AGG AAT TCC ATG AGT GAG GCC CGC AGG---3'

Primer 7: 1024Cla400Dn (30-mer, w/ 1293-1310 nt of ICT1024 gene, GenBank Accession Number: BC014425) (SEQ ID NO:67)

5'---CGC GGC ATC GAT GTC CAT GTC CTC GAT CTG---3'

5 PCR reaction using Primer 1/ Primer 7 and MGC 20194 template generated a 1221 bp DNA fragment that contains the 111 to 1310 nt of the ICT1024. After purification of the PCR products, the 1221 bp DNA fragment was digested with EcoRI and Cla I, and then cloned into pETBlue-2 vector cleaved with EcoRI and Cla I. The final product, pETBlue-2-ICT1024N plasmid DNA, was identified and its sequence was confirmed by DNA sequencing.

10 See Fig. 17 for the restriction map of pETBlue-2-ICT1024N. See Fig. 31 for the sequence. (SEQ ID NO:68)

Example 15. Cloning of the cDNA fragment coding the C terminus peptide (372 aa) of ICT1024 into pETBlue-2 vector for protein expression in E.coli

15 The cDNA coding for the C-terminal 372 aa of ICT1024 was generated by a PCR amplification using a MGC20194 DNA as template. One pair of primers was used to generate the 1139 bp cDNA fragment containing a EcoRI site at its 5' and a Cla I site at its 3' end. No stop codon was integrated into the end of coding region since there is a stop codon downstream of the pETBlue-2 vector.

20 Primer 6: 1024midEcoUp (30-mer, with EcoRI site 1560-1577 nt of ICT1024 gene, GenBank Accession Number: BC014425) (SEQ ID NO:63)

5'--- CCC AGG AAT TCC CAG GTG CAC AGC TTC ATT---3'

Primer 8: 1024ClaDn (30-mer, corresponds to 2675-2658 nt of ICT-1024, GenBank Accession Number: BC014425) (SEQ ID NO:65)

5'---CGC GGC ATC GAT GTG GAG CTG AGC GTC CAG---3'

25 PCR reaction using Primer 6/ Primer 3 and MGC 20194 template generated a 1139 bp DNA fragment that contains the 1560 to 2675 nt of the ICT1024. After purification of the PCR products, the 1139bp DNA fragment was digested with EcoRI and Cla I, and then cloned into pETBlue-2 vector cleaved with EcoRI and Cla I. The final product, pETBlue-2-ICT1024C plasmid DNA, was identified and its sequence was confirmed by DNA sequencing.

30 See Fig. 18 for restriction map of pETBlue-2-ICT1024C plasmid. See Fig. 32 for the sequence. (SEQ ID NO:69)

Production and purification of ICT1024 protein and peptides

Purified ICT1024 protein or peptides are required as antigen for generating ICT1024 specific antibodies using conventional methods. The ICT1024 protein or peptides can be produced from various expression systems that include, but not limit to, mammalian culture
5 cells, yeast, insect cells, and E.coli cells. Only purification methods that preserve protein antigenicity be used for generating ICT1024 protein or peptides. In general, the first step is to introduce the expression vectors carrying a full-length 1024 cDNA or a fragment of cDNA coding for ICT1024 peptide into their corresponding host systems. For example, the mammalian expression vectors are introduced into 293 cells using standard transfection
10 procedures such as liposome mediated or electroporation mediated transfection. The second step is to amplify the host cells carrying the expression vector. One example is the fermentation of yeast or E.coli host cells transformed with the expression vector. The third step is to induce the expression of recombinant protein in the host cells, if inducible expression vector is used. This is particularly important if the recombinant protein is toxic to
15 the host cells. The next step is to isolated recombinant protein from the host cells lysate. The final step is to remove the fusion domain and purify the desired recombinant protein or peptide, if the recombinant protein was generated in the form of a fusion protein.

Example 16. Production of ICT1024 protein or peptide using pGEX-5X-3 /BL21 cells

The Glutathione S-transferase (GST) Gene Fusion System (Amersham) is a versatile
20 system for the expression, purification, and detection of fusion proteins produced in Eschericia coli. The system provides an inducible, high-level expression of genes or gene fragments as fusions with GST, with GST moiety at the amino terminus and the protein of interest at the carboxyl terminus. GST fusion proteins are purified from bacterial lysate by affinity chromatography using immobilized glutathione. GST fusion proteins are captured by
25 the glutathione medium and the impurities are removed by washing. The reduced glutathione is used to elute fusion proteins under mild, non-denaturing conditions to preserve protein's tumorigenicity. For generating ICT1024 protein or peptides as antigen for production of antibodies, the ICT1024 protein or peptides is cleaved from GST using a site-specific protease whose recognition sequence is located immediately upstream from the multiple
30 cloning site on the pGEX plasmids.

Screening for proper GST expression colonies

The pGEX-5X-3-ICT1024, pGEX-5x-3-1024N, or pGEX-5x-3-ICT1024C is transformed into host cell E.coli BL21 using standard protocol provided by Amersham.

Inoculate 12 single colonies into 2 ml in LB medium with 100ug/ml ampicillin, incubate at 37oC with shaking (250 rpm) until OD595 reach 0.6.

- 5 Split the culture into two 1 ml for IPTG induction (I) and un-induction (NI). To "I" tube, add IPTG to a final concentration of 0.1-0.5 mM.

Continue incubation at 37oC with shaking (250 rpm) for 3 hours then transfer culture to a 1.5 ml micro-tube, recover cells by centrifugation at 14,000 rpm for 1 minute.

- 10 Add 200 ul of Protein Sample Buffer to the cell pellet, suspend cells, then boil sample at 100oC for 2-5 minutes.

Load 15 ul of each sample onto a 10% SDS-PAGE gel, with protein molecular weigh markers on parallel lane. After running the gel, stain the gel with coomassie blue. The molecular weigh for GST protein alone is 26Kd, for fusion protein of GST-ICT1024 is 122kd, for fusion protein of GST-ICT1024N is 88 Kd, for fusion protein of GST-ICT1024C is 57 Kd.

- 15 Individual clones with highest expression levels of GST fusion protein are selected for production of respect GST fusion protein.

Isolation of GST-fusion protein

Inoculate a selected single colony into 100 ml LB with 100 ug/ml ampicillin, incubate at 37oC with shaking (250 rpm) overnight.

- 20 Transfer 25 ml overnight culture into 1 liter pre-warmed LB with 100 ug/ml ampicillin in a 2-L flask, incubate at 37oC with shaking (250 rpm) until OD595 reach 0.6.

Add IPTG to a final concentration of 0.1-0.5 mM to induce GST fusion protein expression, incubate at 37oC with shaking (250 rpm) for 3 hours.

Harvest cells by centrifugation at 3,600 rpm for 10 minutes (Servall GS-3 rotor).

- 25 Resuspend cells in 20 ml R.S. with protease inhibitor (see appendix for R.S. buffer and R.S. buffer cocktail).

Sonicate sample for 6 times, 30 seconds each. Keep sample on ice during the sonication and mix the sample after each sonication.

- 30 Centrifuge the sonicated sample at 10,000 rpm for 10 minutes using a Servall GS-3 rotor. Transfer the supernatant to a fresh tube.

To the supernatant add glutathione-agarose beads slurry (GSH-Agarose powder, sigma G4510, 70 mg beads balanced in 4 ml RS, inversed at RT in a 15-ml tube for 1 hr to overnight, with 2-3 buffer replacements, resulting in 1 ml compact swollen beads slurry,

could be stored as 50% slurry for a month. Use material from 1.5-2.0 liters of supernatant (37.5-50 ml) per 1 ml of resin in 50 ml tube.)

Mix the super/slurry gently at 40C on rotator for 0.5-2 hr.

Centrifuge at 1,500 rpm for 2 minutes, batch wash 2 times with 10 ml RS each. Add
5 10 ml RS to suspend sample, load onto a column.

Rinse the column with 10 ml RS.

Strip the column with 50 mM GSH in RS (pH 8.0, adjusted by NaOH).

Collect 0.5 ml fractions by hand or a fraction collector. GST fusion protein should be eluted in fractions 5-11.

10 Locate GST fusion protein by placing 2 ul aliquots of each fraction into wells of microplates and adding 100 ul of 1x Bradford reagent (1:5 dilutions of Biorad reagent) and check the color of mixture.

To remove the GST from ICT1024 protein or peptides, the mixture of super/slurry, after batch wash, is washed 1x with thrombin cleavage buffer (T.C.B., see appendix). Then,
15 add 2 ml T.C.B. and 10 µg (13 ul) of Thrombin (0.768 µg/µl), shaking at RT for 1.5 hours. (Thrombin, human, lyoph, Cat: 605195, Calbiochem Corp.)

Appendix:

20 R.S. buffer (500 ml)

1M Tris.HCl, pH 7.9	10 ml (20 mM)
0.5 M EDTA	0.2 ml (0.2 mM)
NaCl	29.22 g (1 M)

R.S. buffer cocktail

R.S. plus the following	500 ml	1 liter	Final conc.
2ME (40C)	1 ml	2 ml	0.2%
NP-40 (RT)	2.5 ml	5 ml	0.5%
PMSF (RT) MW174.2	17.42 mg (in EtOH)	34.84 mg	0.2 mM
Aprotinin (-200C, H ₂ O)	1 mg (freely in water)	2 mg	2 µg/ml

Leupeptin (40C or -200C)	1 mg (in water, 1 mg/ml)	2 mg	2 µg/ml
Pepstatin-A (40C or -200C)	1 mg (in EtOH, up to 1 mg/ml)	2 mg	2 µg/ml

T.C.B. (Thrombin cleavage buffer), for 30 ml:

1 M Tris, pH 8.0	1.5 ml (1:20. 50 mM)
4 M NaCl	1.125 ml (0.15 M)
1 M CaCl ₂	0.075ml (2.5 mM)
2ME	0.03 ml (0.1%)

Example 17. Production of ICT1024 protein or peptide using pETBlue-2/BL21 cells

5
ICT1024 is a membrane-associated proteinase belongs to the RHO family. Our preliminary data in pGEX-5X-3/BL21 expression system indicated that the GST-ICT1024 fusion protein is very toxic to the bacterial host, and therefore it is difficult to get the high levels of GST-ICT1024 fusion protein expression by IPTG induction in DH5 α -T1 or BL21
10 cells. The pETBlue system (Novagen) may likely help us to solve the toxicity problem. The pET-Blue2 vector employs the bacteriophage T7 promoter to drive the expression of the interested gene. The bacteriophage T7 polymerase only will be expressed in BL(DE3) cells when induced by IPTG. When BL21(DE3)pLysS cells are used, the T7 polymerase activity will be further contained by the expressed T7 lysozyme. All these features of this system
15 make the expression of the interested protein very selective and tightly controlled, that favors my present purpose: to express otherwise very toxic proteins. Another advantage of using the pETBlue-2 is the utilization of α -complementation of LacZ gene product, β -Galactosidase, to use blue/white colony based selection of plasmid constructs. Additionally, the C'-end of the engineered fusion protein contains the in-frame tags: HSV Tag and His.Tag that are linked in
20 tandem. These tags can be used for purification and detection of the fusion products, respectively.

The pETBlue-2-ICT1024, pETBlue-2-1024N, or pETBlue-2-ICT1024C plasmid DNA is transformed into host cell E.coli BL21 using standard protocol provided by Novagen. The transformed clones are easily visual identified by blue/white colony screening, since

pETBlue-2 vector uses a weak constitutive E. coli promoter (tet) to drive expression of the lacZ alpha-peptide, whereas expression of ICT1024 gene is driven by a T7lac promoter in the opposite orientation. Insertion of ICT1024 sequences into the multiple cloning site (MCS) disrupts expression of the lacZ alpha-peptide and produces a white colony phenotype in strain DH5a when plated in the presence of X-gal. Colonies derived from the unmodified vector turn blue. Because T7-driven protein expression requires inserts to be cloned in an antisense orientation relative to the tet promoter, basal expression of ICT1024 sequences is virtually absent. The high copy number pUC origin of replication present on the pETBlue-2 plasmids greatly increases plasmid yields and therefore the expressed ICT1024 protein or peptides.

The ICT1024 gene or fragments in pETBlue-2 vector are expressed at high levels, because the inserted sequences are in the sense orientation relative to the T7lac promoter, and the reading frame meet the translation requirements of pETBlue-2 vector. Protein expression is accomplished by transforming the recombinant pETBlue-2 plasmids into the host strains Tuner™(DE3)pLacI or Origami™(DE3)pLacI followed by induction with IPTG. These hosts carry a chromosomal copy of the T7 RNA polymerase gene under lacUV5 control, and supply sufficient lac repressor via the compatible pLacI plasmid to ensure low level uninduced expression. The lacY status of the Tuner strain allows uniform dose-dependent IPTG induction of the target protein throughout the culture, and Origami strains enhance cytoplasmic disulfide bond formation.

Furthermore, since the ICT1024, ICT1024N, and ICT1024C inserts all lack an internal stop codon and were cloned in-frame with the C-terminal HSV-Tag® epitope and His-Tag® sequences. The ICT1024 protein or peptides are expressed in the form of fusion protein with HSV Tag and His Tag at its C-terminal. The ICT1024 protein and peptides are isolated and purified following Novagen's standard procedure.

Example 18. Production of ICT1024 protein or peptide using 293 cells

Even through mild, non-denaturing conditions were used for purify recombinant proteins from E. coli to preserve their antigenicity, too many times that the purified protein lost their antigenicity due to lower solubility or unsatisfied un-folding of the recombinant protein. Utilizing mammalian culture system for expression recombinant protein can overcome this hurdle, though the yield of recombinant protein from such a system usually is much lower than the E.coli expression system.

I. Transfection of HEK 293 cells using Electroporation approach

Grow cells in RPMI 1640 medium containing 10% FBS.

Wash cells with FBS free RPMI 1640 media, add trypsin;

5 Inactivate trypsin with RPMI 1640 medium containing 10% FBS.

Wash cells times using RPMI 1640 media with 2.5% FBS (no antibiotics).

Resuspend the cells in RPMI 1640 media with 2.5% FBS at a density of 5×10^6 cells/ml.

10 Transfer 200 μ l cells into an sterile electroporation cuvette (BTX Cuvettes Model #620: 2 mm gap). Add 10 μ g of plasmide DNA (pCI-ICT1024, pCI-ICT1024N, or pCI-ICT1024C) into the cuvettes and mix well. Incubate cells and DNA for 10 minutes at room temperature before electroporation.

Electroporation Settings:

15

Electroporation generator	BTX ECM 830
Voltage (V)	1200 HV
Pulse Length (μ s)	50 μ s
No. of pulses (n)	1

20

After electroporation, let the cells recover for 10 minute incubation at room temperature.

25 Place the transfected cells (1×10^6) into in a single well of a 6-well plate containing 2 ml of prewarmed RPMI medium with 10% serum, and incubate in 37°C, 5% CO₂ incubator for 48 hours.

II. Protein extraction from cell membrane

30 Since ICT1024 protein is a membrane associated protein with majority of its C terminus residues in the membrane, the ICT1204 protein and the C terminus peptides expressed in the transfected cells need to be extracted from cell memberane using a M-PER Eukaryotic membrane Protein Extraction Reagent kit(Cat No: 89826, PIERCE).

Isolate 5×10^6 cells per sample by centrifuging harvested cell suspensions at $850 \times g$ for 2 minutes. Pellet cells (washed in PBS) in 1.7 ml conical microcentrifuge tubes.

Carefully remove and discard the supernatant.

5 Add 150 μ l of Reagent A to the cell pellet. Pipette up and down to obtain a homogeneous cell suspension. Incubate 10 minutes at room temperature with occasional vortexing.

Place lysed cells on ice.

10 Dilute 2 parts Reagent C with 1 part Reagent B, making sufficient mixture for each sample to receive 450 μ l (e.g., for 10 extractions, combine 3.33 ml of Reagent C with 1.67 ml of Reagent B). Note: Keep Reagent C at 4°C or on ice at all times.

Add 450 μ l of diluted Reagent C to each tube of lysed cells and vortex. Incubate tubes on ice for 30 minutes, vortexing every 5 minutes.

Centrifuge tubes at $10,000 \times g$ for 3 minutes at 4°C . Transfer supernatant to new tubes.

Incubate supernatant for 10 minutes at 37°C to separate the membrane protein fraction.

15 Centrifuge tubes at room temperature for 2 minutes at $10,000 \times g$ to isolate the hydrophobic fraction (i.e., the fraction containing membrane protein of interest) from the hydrophilic fraction.

Carefully remove the hydrophilic phase (top layer) from the hydrophobic protein phase (bottom layer) and save in a new tube. Perform the phase separations as quickly as possible because the interface between the layers slowly disappears at room temperature. Place the separated fractions on ice.

Note: The majority of membrane protein should be found in the lower viscous phase.

Note: The hydrophobic fraction can now be used for membrane protein(s) analysis.

25 III. Protein extraction from whole cells

The N terminus peptides of ICT1024 is likely not tightly associated with the cell membrane, therefore, it is much easy to isolated from the cell lysates using a M-PER Mammalian Protein Extraction Reagent (Cat No: 78501, PIERCE)

Carefully remove (decant) culture medium from the adherent cells.

30 Wash the cells once with PBS.

Add an 300 ul of M-PER™ Reagent to each plate well (6-well plate).

Shake gently for 5 minutes.

Collect the lysate and transfer to a microcentrifuge tube.

Centrifuge samples at 27,000g for 5-10 minutes to pellet the cell debris.

Transfer supernatant to a clean tube for further analysis (SDS page or western blotting).

IV. Isolation of ICT1024 protein or peptides from SDS-Page gel

5 Dilute the protein sample 1:1 with 2x SDS Sample Buffer, heat the samples and the molecular weight standards for 5 minutes at 100°C.

The samples are loaded onto a 10% SDS-Page gel.

Run the gel at 10 mA until the dye enters the separating gel. Then increase the current to 15 mA. When the dye reaches the bottom of the separating gel, turn off the power supply,
10 and remove the gel sandwich.

Carefully open the sandwich by using one of the spacers to pry the plates apart. Gently cut away the stacking gel and place the separating gel in a small plastic container for staining.

Cover the gel with fixing solution and shake gently for 15 minutes.

15 Pour off the fixer and cover the gel with Coomassie blue staining solution. Shake gently for at least 2 hours. Pour off the staining solution and cover the gel with the wash solution.

Cut of the gel fragment containing the desired protein band, extract protein from the gel using standard procedures.

20 Example 19 Production of ICT1024 antibodies

The purified ICT1024 protein or peptides are used to generate ICT1024 antibodies. The mammalian expression vectors carrying ICT1024 full-length cDNA or fragment are used to generate ICT1024 antibodies directly using DNA vaccination methods. In addition, a series of ICT1024 peptides (15 aa to 30 aa) will be chemically synthesized as antigen for
25 generating ICT1024 antibodies. Further more, since ICT1024 is a membrane protein, plasmid DNA will be constructed for expression of ICT1024 specific intrabodies (single-chain Fv fragment, scFv) within the cell and directed against ICT1024's intracellular domains.

The ICT1024 antibodies to be generated include, but not limit to, mouse polyclonal antibodies, mouse monoclonal antibodies (MAb), rabbit polyclonal antibodies, rabbit
30 monoclonal antibodies, chicken IgY antibodies, and humanized antibodies.

Example 20. Generation of ICT1024 antibodies by directly DNA vaccination of mouse

Plasmid DNA or polynucleotides have been proved to be good alternative vaccines to traditional whole organism or purified proteins. Advantages of DNA vaccination over traditional methods are listed below:

5 Simple: subcloning of DNA sequence into vectors (plasmid, or viral) is much easier than tedious and often very difficult undertakes of purification of antigen proteins.

 Safer: individual proteins pose little risk of causing infection. If specific epitope sequences are selected for vaccination the toxicity, if any, of natural proteins could be also minimized as well.

10 Natural: studies showed that antigens (proteins or polypeptides) produced in situ from DNA vaccines would adopt a natural conformation and have necessary post-translation modifications made by host during natural infections.

 Although enhanced immune responses have been reported when DNA vaccines are delivered with cationic lipids, gene gun, or jet injection, the electroporation is by far the most
15 efficient way for DNA transfection both in vitro and in vivo. The combination of plasmid DNA injection and electroporation delivery has produced convincing positive results on different tissues, such as muscle, skin, tumor xenografts, etc.

 As the combination of DNA vaccine and electroporation offers a convenient and speedy way to generate polyclonal antibodies in mice, this approach can thus be used to
20 screen for potential antibody targets discovered in house which may have applications in disease diagnosis, or treatment.

Procedure

 After Balb/c mouse is anesthetized, a stripe of skin on the back of mouse is shaved to
25 expose the area of skin. Five location on one mouse were shaved. Two ug of pCI-ICT1024, pCI-ICT1024N, or pCI-ICT1024C plasmid DNA in 20μl normal saline is injected into layer of skin of in each shaved area via route of subcutaneous using a 1-ml syringe and a 30.5 gauge needle. Electroporations are then applied immediately on the injected area after the injection with parameters set as: Voltage= 100V, Pulse Length= 20ms, Pulse Number= 3, and
30 Pulse Interval= 800ms.

 The DNA vaccination (immunization) procedure was repeated 7 days later, and repeated another time 1 month later. Blood samples are collected 7 days after the last DNA vaccination for testing of immunization effectiveness. In other experiments, the last boosting

was also achieved by injecting lysates of mouse or human tumor cells that are transfected beforehand with the same DNA.

The effectiveness of immunization with DNA expression were tested with ELISA, Western, or functional assays like cell proliferation assay, apoptosis assay. For ELISA assay
5 plastic support surface were coated with crude lysates from transfected cos-7 cells or 293 cells as source of antigen, which is then detected with antibody present in the immunoglobulins purified from the sera of immunized mice. In other experiment, the antisera collected from immunized mice were used to precipitate ICT1024 protein or peptides (antigens) presented in the lysates of transfected cos-7 or 203 cells. The precipitated targets
10 are then detected by Western Blotting.

Specific antibodies against ICT1024 can be purified using traditional methods, DEAE ion-exchange column, Protein-A affinity column, etc. Pure antibodies can be obtained after monoclonal antibodies are produced through hybridoma technology.

Example 21. Generation of rabbit monoclonal antibodies against ICT1024

15 To generate rabbit monoclonal antibodies against ICT1024, the expression vectors carrying ICT1024 full-length cDNA or cDNA fragment are transfected into the rabbit cell line 240E. The resulting transfected cells are pooled and used to immunize a rabbit. Endogenous proteins from the cell line 240E do not induce immune reaction and only the expressed human proteins are recognized as antigens by the rabbit. The combination of high
20 fusion efficiency, better stability of hybridoma and a large repertory of antibody-producing cells make it possible to multiplex antigens for immunizing a single rabbit.

Example 22. Generation of mouse monoclonal antibodies against ICT1024 using ICT1024protein or peptides as antigen

Coventional methods will be employed to generate and purify mouse polyclonal
25 antibodies and monoclonal antibodies against ICT1024 protein. We will also utilize chemically synthesized ICT1024 peptides corresponding to different domains of ICT1024 protein to generate and purify mouse polyclonal antibodies and monoclonal antibodies against ICT1024 protein. The goal of this is to screen the best monoclonal antibodies against ICT1024, indicated by high affinity of binding with ICT1024 protein and more importantly,
30 the capability of block the biological function of ICT1024 protein through antibody/antigen specific binding.

Example 23. Cloning of ICT1025 full-length cDNA into pCI vector for mammalian cell expression and DNA vaccination

The full-length cDNA of ICT1025 (803 aa) was generated by PCR amplification using a cDNA clone purchased from ATCC (MGC: 20194) as template. Since the full-length
5 of ICT1025 cDNA is 2780 bp, to reduce the mutation may occur during the PCR reaction, two pairs of primers were designed to generate two shorter DNA fragments that can be legated together to generate full-length ICT1025 cDNA.

See Fig. 20. The confirmed sequence of pCI-ICT1025 expression plasmid.

10 Production and purification of ICT1025 protein and peptides

Purified ICT1025 protein or peptides are required as antigen for generating ICT1025 specific antibodies using conventional methods. The ICT1025 protein or peptides can be produced from various expression systems that include, but not limit to, mammalian culture cells, yeast, insect cells, and E.coli cells. Only purification methods that preserve protein
15 antigenicity be used for generating ICT1025 protein or peptides. In general, the first step is to introduce the expression vectors carrying a full-length ICT1025 cDNA or a fragment of cDNA coding for ICT1025 peptide into their corresponding host systems. For example, the mammalian expression vectors are introduced into 293 cells using standard transfection procedures such as liposome mediated or electroporation mediated transfection. The second
20 step is to amplify the host cells carrying the expression vector. One example is the fermentation of yeast or E.coli host cells transformed with the expression vector. The third step is to induce the expression of recombinant protein in the host cells, if inducible expression vector is used. This is particularly important if the recombinant protein is toxic to the host cells. The next step is to isolated recombinant protein from the host cells lysate. The
25 final step is to remove the fusion domain and purify the desired recombinant protein or peptide, if the recombinant protein was generated in the form of a fusion protein.

Example 24. Production of ICT1025 protein or peptide using pGEX-5X-3 /BL21 cells

The Glutathione S-transferase (GST) Gene Fusion System (Amersham) is a versatile system for the expression, purification, and detection of fusion proteins produced in
30 Eschericia coli. The system provides an inducible, high-level expression of genes or gene fragments as fusions with GST, with GST moiety at the amino terminus and the protein of interest at the carboxyl terminus. GST fusion proteins are purified from bacterial lysate by affinity chromatography using immobilized glutathione. GST fusion proteins are captured by

the glutathione medium and the impurities are removed by washing. The reduced glutathione is used to elute fusion proteins under mild, non-denaturing conditions to preserve protein's tumorigenicity. For generating ICT1025 protein or peptides as antigen for production of antibodies, the ICT1025 protein or peptides is cleaved from GST using a site-specific
5 protease whose recognition sequence is located immediately upstream from the multiple cloning site on the pGEX plasmids.

Screening for proper GST expression colonies

The pGEX-5X-3-ICT1024, pGEX-5x-3-1024N, or pGEX-5x-3-ICT1024C is
10 transformed into host cell E.coli BL21 using standard protocol provided by Amersham.

Inoculate 12 single colonies into 2 ml in LB medium with 100ug/ml ampicillin, incubate at 37oC with shaking (250 rpm) until OD595 reach 0.6.

Split the culture into two 1 ml for IPTG induction (I) and un-induction (NI). To "I" tube, add IPTG to a final concentration of 0.1-0.5 mM.

15 Continue incubation at 37oC with shaking (250 rpm) for 3 hours then transfer culture to a 1.5 ml micro-tube, recover cells by centrifugation at 14,000 rpm for 1 minute.

Add 200 ul of Protein Sample Buffer to the cell pellet, suspend cells, then boil sample at 100oC for 2-5 minutes.

Load 15 ul of each sample onto a 10% SDS-PAGE gel, with protein molecular weigh
20 markers on parallel lane. After running the gel, stain the gel with coomassie blue. The molecular weigh for GST protein alone is 26Kd, for fusion protein of GST-ICT1025 is 122kd, for fusion protein of GST-ICT1024N is 38 Kd, for fusion protein of GST-ICT1024C is 57 Kd.

Individual clones with highest expression levels of GST fusion protein are selected for production of respect GST fusion protein.

25

Isolation of GST-fusion protein

Inoculate a selected single colony into 100 ml LB with 100 ug/ml ampicillin, incubate at 37oC with shaking (250 rpm) overnight.

Transfer 25 ml overnight culture into 1 liter pre-warmed LB with 100 ug/ml
30 ampicillin in a 2-L flask, incubate at 37oC with shaking (250 rpm) until OD595 reach 0.6.

Add IPTG to a final concentration of 0.1-0.5 mM to induce GST fusion protein expression, incubate at 37oC with shaking (250 rpm) for 3 hours.

Harvest cells by centrifugation at 3,600 rpm for 10 minutes (Servall GS-3 rotor).

Resuspend cells in 20 ml R.S. with protease inhibitor (see appendix for R.S. buffer and R.S. buffer cocktail).

Sonicate sample for 6 times, 30 seconds each. Keep sample on ice during the sonication and mix the sample after each sonication.

- 5 Centrifuge the sonicated sample at 10,000 rpm for 10 minutes using a Servall GS-3 rotor. Transfer the supernatant to a fresh tube.

To the supernatant add glutathione-agarose beads slurry (GSH-Agarose powder, sigma G4510, 70 mg beads balanced in 4 ml RS, inversed at RT in a 15-ml tube for 1 hr to overnight, with 2-3 buffer replacements, resulting in 1 ml compact swollen beads slurry, 10 could be stored as 50% slurry for a month. Use material from 1.5-2.0 liters of supernatant (37.5-50 ml) per 1 ml of resin in 50 ml tube.)

Mix the super/slurry gently at 40C on rotator for 0.5-2 hr.

Centrifuge at 1,500 rpm for 2 minutes, batch wash 2 times with 10 ml RS each. Add 10 ml RS to suspend sample, load onto a column.

- 15 Rinse the column with 10 ml RS.

Stripe the column with 50 mM GSH in RS (pH 8.0, adjusted by NaOH).

Collect 0.5 ml fractions by hand or a fraction collector. GST fusion protein should be eluted in fractions 5-11.

- 20 Locate GST fusion protein by placing 2 ul aliquots of each fraction into wells of microplates and adding 100 ul of 1x Bradford reagent (1:5 dilutions of Biorad reagent) and check the color of mixture.

- To remove the GST from ICT1025 protein or peptides, the mixture of super/slurry, after batch wash, is washed 1x with thrombin cleavage buffer (T.C.B., see appendix). Then, add 2 ml T.C.B. and 10 µg (13 ul) of Thrombin (0.768 µg/µl), shaking at RT for 1.5 hours. 25 (Thrumbin, human, lyoph, Cat: 605195, Calbiochem Corp.).

Example 25. Production of ICT1025 protein or peptide using pETBlue-2/BL21 cells

- ICT1025 is a membrane-associated proteinase belongs to the RHO family. Our preliminary data in pGEX-5X-3/BL21 expression system indicated that the GST-ICT1025 fusion protein is very toxic to the bacterial host, and therefore it is difficult to get the high 30 levels of GST-ICT1025 fusion protein expression by IPTG induction in DH5α-T1 or BL21 cells. The pETBlue system (Novagen) may likely help us to solve the toxicity problem. The pET-Blue2 vector employs the bacteriophage T7 promoter to drive the expression of the

interested gene. The bacteriophage T7 polymerase only will be expressed in BL(DE3) cells when induced by IPTG. When BL21(DE3)pLysS cells are used, the T7 polymerase activity will be further contained by the expressed T7 lysozyme. All these features of this system make the expression of the interested protein very selective and tightly controlled, that favors my present purpose: to express otherwise very toxic proteins. Another advantage of using the pETBlue-2 is the utilization of α -complementation of LacZ gene product, β -Galactosidase, to use blue/white colony based selection of plasmid constructs. Additionally, the C'-end of the engineered fusion protein contains the in-frame tags: HSV Tag and His.Tag that are linked in tandem. These tags can be used for purification and detection of the fusion products, respectively.

The pETBlue-2-ICT1025, pETBlue-2-1025N, or pETBlue-2-ICT1025C plasmid DNA is transformed into host cell E.coli BL21 using standard protocol provided by Novagen. The transformed clones are easily visual identified by blue/white colony screening, since pETBlue-2 vector uses a weak constitutive E. coli promoter (tet) to drive expression of the lacZ alpha-peptide, whereas expression of ICT1025 gene is driven by a T7lac promoter in the opposite orientation. Insertion of ICT1025 sequences into the multiple cloning site (MCS) disrupts expression of the lacZ alpha-peptide and produces a white colony phenotype in strain DH5a when plated in the presence of X-gal. Colonies derived from the unmodified vector turn blue. Because T7-driven protein expression requires inserts to be cloned in an antisense orientation relative to the tet promoter, basal expression of ICT1025 sequences is virtually absent. The high copy number pUC origin of replication present on the pETBlue-2 plasmids greatly increases plasmid yields and therefore the expressed ICT1025 protein or peptides.

The ICT1025 gene or fragments in pETBlue-2 vector are expressed at high levels, because the inserted sequences are in the sense orientation relative to the T7lac promoter, and the reading frame meet the translation requirements of pETBlue-2 vector. Protein expression is accomplished by transforming the recombinant pETBlue-2 plasmids into the host strains Tuner™(DE3)pLacI or Origami™(DE3)pLacI followed by induction with IPTG. These hosts carry a chromosomal copy of the T7 RNA polymerase gene under lacUV5 control, and supply sufficient lac repressor via the compatible pLacI plasmid to ensure low level uninduced expression. The lacY status of the Tuner strain allows uniform dose-dependent IPTG induction of the target protein throughout the culture, and Origami strains enhance cytoplasmic disulfide bond formation. Refer to Figure 19 for ICT1025 protein purified with above-described procedures.

Furthermore, since the ICT1025, ICT1025N, and ICT1025C inserts all lack an internal stop codon and were cloned in-frame with the C-terminal HSV•Tag® epitope and His•Tag® sequences. The ICT1025 protein or peptides are expressed in the form of fusion protein with HSV Tag and His Tag at its C-terminal. The ICT1025 protein and peptides are isolated and purified following Novagen's standard procedure.

Example 26. Production of ICT1025 protein or peptide using 293 cells

Even through mild, non-denaturing conditions were used for purify recombinant proteins from E. coli to preserve their antigenicity, too many times that the purified protein lost their antigenicity due to lower solubility or unsatisfied un-folding of the recombinant protein. Utilizing mammalian culture system for expression recombinant protein can overcome this hurdle, though the yield of recombinant protein from such a system usually is much lower than the E.coli expression system.

I. Transfection of HEK 293 cells using Electroporation approach

Grow cells in RPMI 1640 medium containing 10% FBS.

Wash cells with FBS free RPMI 1640 media, add trypsin;

Inactivate trypsin with RPMI 1640 medium containing 10% FBS.

Wash cells times using RPMI 1640 media with 2.5% FBS (no antibiotics).

Resuspend the cells in RPMI 1640 media with 2.5% FBS at a density of 5x10⁶ cells/ml.

Transfer 200 ul cells into an sterile electroporation cuvette (BTX Cuvettes Model #620: 2 mm gap). Add 10 ug of plasmide DNA (pCI-ICT1025, pCI-ICT1025N, or pCI-ICT1025C) into the cuvettes and mix well. Incubate cells and DNA for 10 minutes at room temperature before electroporation.

Electroporation Settings:

Electroporation generator	BTX ECM 830
Voltage (V)	1200 HV
Pulse Length	50 μ s
No. of pulses (n)	1

After electroporation, let the cells recover for 10 minute incubation at room temperature.

Place the transfected cells (1×10^6) into in a single well of a 6-well plate containing 2 ml of prewarmed RPMI medium with 10% serum, and incubate in 37°C, 5% CO₂ incubator for 48 hours.

II. Protein extraction from cell membrane

Since ICT1025 protein is a membrane associated protein with majority of its C terminus residues in the membrane, the ICT1205 protein and the C terminus peptides expressed in the transfected cells need to be extracted from cell memberane using a M-PER Eukaryotic membrane Protein Extraction Reagent kit(Cat No: 89826, PIERCE).

Isolate 5×10^6 cells per sample by centrifuging harvested cell suspensions at $850 \times g$ for 2 minutes. Pellet cells (washed in PBS) in 1.7 ml conical microcentrifuge tubes.

Carefully remove and discard the supernatant.

Add 150 μ l of Reagent A to the cell pellet. Pipette up and down to obtain a homogeneous cell suspension. Incubate 10 minutes at room temperature with occasional vortexing.

Place lysed cells on ice.

Dilute 2 parts Reagent C with 1 part Reagent B, making sufficient mixture for each sample to receive 450 μ l (e.g., for 10 extractions, combine 3.33 ml of Reagent C with 1.67 ml of Reagent B). Note: Keep Reagent C at 4°C or on ice at all times.

Add 450 μ l of diluted Reagent C to each tube of lysed cells and vortex. Incubate tubes on ice for 30 minutes, vortexing every 5 minutes.

Centrifuge tubes at $10,000 \times g$ for 3 minutes at 4°C. Transfer supernatant to new tubes.

Incubate supernatant for 10 minutes at 37°C to separate the membrane protein fraction.

Centrifuge tubes at room temperature for 2 minutes at $10,000 \times g$ to isolate the hydrophobic fraction (i.e., the fraction containing membrane protein of interest) from the hydrophilic fraction.

Carefully remove the hydrophilic phase (top layer) from the hydrophobic protein phase (bottom layer) and save in a new tube. Perform the phase separations as quickly as possible because the interface between the layers slowly disappears at room temperature. Place the separated fractions on ice.

Note: The majority of membrane protein should be found in the lower viscous phase.

Note: The hydrophobic fraction can now be used for membrane protein(s) analysis.

III. Protein extraction from whole cells

The N terminus peptides of ICT1025 is likely not tightly associated with the cell
5 membrane, therefore, it is much easy to isolated from the cell lysates using a M-PER
Mammalian Protein Extraction Reagent (Cat No: 78501, PIERCE)

Carefully remove (decant) culture medium from the adherent cells.

Wash the cells once with PBS.

Add an 300 ul of M-PER™ Reagent to each plate well (6-well plate).

10 Shake gently for 5 minutes.

Collect the lysate and transfer to a microcentrifuge tube.

Centrifuge samples at 27,000g for 5-10 minutes to pellet the cell debris.

Transfer supernatant to a clean tube for further analysis (SDS page or western
blotting).

15

IV. Isolation of ICT1025 protein or peptides from SDS-Page gel

Dilute the protein sample 1:1 with 2x SDS Sample Buffer, heat the samples and the
molecular weight standards for 5 minutes at 100°C.

The samples are loaded onto a 10% SDS-Page gel.

20 Run the gel at 10 mA until the dye enters the separating gel. Then increase the current
to 15 mA. When the dye reaches the bottom of the separating gel, turn off the power supply,
and remove the gel sandwich.

Carefully open the sandwich by using one of the spacers to pry the plates apart.
Gently cut away the stacking gel and place the separating gel in a small plastic container for
25 staining.

Cover the gel with fixing solution and shake gently for 15 minutes.

Pour off the fixer and cover the gel with Coomassie blue staining solution. Shake
gently for at least 2 hours. Pour off the staining solution and cover the gel with the wash
solution.

30 Cut of the gel fragment containing the desired protein band, extract protein from the
gel using standard procedures.

Example 27 Production of ICT1025 antibodies

The purified ICT1025 protein or peptides are used to generate ICT1025 antibodies. The mammalian expression vectors carrying ICT1025 full-length cDNA or fragment are used to generate ICT1025 antibodies directly using DNA vaccination methods. In addition, a series of ICT1025 peptides (15 aa to 30 aa) will be chemically synthesized as antigen for generating ICT1025 antibodies. Further more, since ICT1025 is a membrane protein, plasmid DNA will be constructed for expression of ICT1025 specific intrabodies (single-chain Fv fragment, scFv) within the cell and directed against ICT1025's intracellular domains.

The ICT1025 antibodies to be generated include, but not limit to, mouse polyclonal antibodies, mouse monoclonal antibodies (MAb), rabbit polyclonal antibodies, rabbit monoclonal antibodies, chicken IgY antibodies, and humanized antibodies.

Example 28. Generation of ICT1025 antibodies by directly DNA vaccination of mouse

Plasmid DNA or polynucleotides have been proved to be good alternative vaccines to traditional whole organism or purified proteins. Advantages of DNA vaccination over traditional methods are listed below:

Simple: subcloning of DNA sequence into vectors (plasmid, or viral) is much easier than tedious and often very difficult undertakes of purification of antigen proteins.

Safer: individual proteins pose little risk of causing infection. If specific epitope sequences are selected for vaccination the toxicity, if any, of natural proteins could be also minimized as well.

Natural: studies showed that antigens (proteins or polypeptides) produced in situ from DNA vaccines would adopt a natural conformation and have necessary post-translation modifications made by host during natural infections.

Although enhanced immune responses have been reported when DNA vaccines are delivered with cationic lipids, gene gun, or jet injection, the electroporation is by far the most efficient way for DNA transfection both in vitro and in vivo. The combination of plasmid DNA injection and electroporation delivery has produced convincing positive results on different tissues, such as muscle, skin, tumor xenografts, etc.

As the combination of DNA vaccine and electroporation offers a convenient and speedy way to generate polyclonal antibodies in mice, this approach can thus be used to screen for potential antibody targets discovered in house which may have applications in disease diagnosis, or treatment.

After Balb/c mouse is anesthetized, a stripe of skin on the back of mouse is shaved to expose the area of skin. Five location on one mouse were shaved. Two ug of pCI-ICT1025, pCI-ICT1025N, or pCI-ICT1025C plasmid DNA in 20µl normal saline is injected into layer of skin of in each shaved area via route of subcutaneous using a 1-ml syringe and a 30.5 gauge needle. Electroporations are then applied immediately on the injected area after the injection with parameters set as: Voltage= 100V, Pulse Length= 20ms, Pulse Number= 3, and Pulse Interval= 800ms.

The DNA vaccination (immunization) procedure was repeated 7 days later, and repeated another time 1 month later. Blood samples are collected 7 days after the last DNA vaccination for testing of immunization effectiveness. In other experiments, the last boosting was also achieved by injecting lysates of mouse or human tumor cells that are transfected beforehand with the same DNA.

The effectiveness of immunization with DNA expression were tested with ELISA, Western, or functional assays like cell proliferation assay, apoptosis assay. For ELISA assay plastic support surface were coated with crude lysates from transfected cos-7 cells or 293 cells as source of antigen, which is then detected with antibody present in the immunoglobulins purified from the sera of immunized mice. In other experiment, the antisera collected from immunized mice were used to precipitate ICT1025 protein or peptides (antigens) presented in the lysates of transfected cos-7 or 203 cells. The precipitated targets are then detected by Western Blotting.

Specific antibodies against ICT1025 can be purified using traditional methods, DEAE ion-exchange column, Protein-A affinity column, etc. Pure antibodies can be obtained after monoclonal antibodies are produced through hybridoma technology.

Example 29. Generation of rabbit monoclonal antibodies against ICT1025

To generate rabbit monoclonal antibodies against ICT1025, the expression vectors carrying ICT1025 full-length cDNA or cDNA fragment are transfected into the rabbit cell line 240E. The resulting transfected cells are pooled and used to immunize a rabbit. Endogenous proteins from the cell line 240E do not induce immune reaction and only the expressed human proteins are recognized as antigens by the rabbit. The combination of high fusion efficiency, better stability of hybridoma and a large repertory of antibody-producing cells make it possible to multiplex antigens for immunizing a single rabbit.

Example 30. Generation of mouse monoclonal antibodies against ICT1025 using ICT1025 protein or peptides as antigen

Conventional methods will be employed to generate and purify mouse polyclonal antibodies and monoclonal antibodies against ICT1025 protein. We will also utilize
5 chemically synthesized ICT1025 peptides corresponding to different domains of ICT1025 protein to generate and purify mouse polyclonal antibodies and monoclonal antibodies against ICT1025 protein. The goal of this is to screen the best monoclonal antibodies against ICT1025, indicated by high affinity of binding with ICT1025 protein and more importantly, the capability of block the biological function of ICT1025 protein through antibody/antigen
10 specific binding.

Generation of hybridomas producing ICT1025 mAb

Mouse monoclonal antibody (mAb) against human ICT1025 protein was generated using a standard procedure. Briefly, the human ICT1025 protein was purified from E.coli cells that transformed with a prokaryotic expression vector for human ICT1025 cDNA. The
15 purified human ICT1025 protein was then used to immunize mouse. After several immunizations, when the ICT1025 antibody titer in the serum of immunized mice exceeded 10,000, the mice were sacrificed and the spleen cells were harvested to generate hybridoma clones. Individual hybridoma clone was then amplified and the culture supernatant was collected for verifying mAb against the ICT1025 protein using an ELISA based assay. From
20 one mouse immunized with ICT1025 protein, 40 hybridoma clones were confirmed to produce mAb specific to human ICT1025 protein.

Selection of ICT1025 mAb with cell surface binding activity

It has been demonstrated that the expression levels of ICT1025 are up-regulated in tumor cells. More importantly, it is believed that migration of ICT1025 protein from
25 cytoplasm to the cell membrane and exposure to the extracellular compartment occurs selectively in tumor cells. Therefore, to be effective for therapeutics or diagnosis, a ICT1025 mAb must be able to bind to the extracellular domain(s) of the ICT1025 protein. A Living Cell Surface Staining ELISA was used to screen the ICT1025 mAb that can bind to the cell surface domain(s) of ICT1025 protein.

30 Two cell lines, a human breast tumor cell line MDA-MB-435 and a human colon tumor cell line HT29, both of them over-expressing ICT1025 protein, were used in the mAb screening studies. The data from mAb screening using hybridoma culture supernatant in MDA-MB-435 cells (Figure 55) and HT29 cells (Figure 56) demonstrated that there are great

variations in surface binding activities among the 40 mAb, with the data from one cell line confirmed the data from the other cell line.

Six hybridoma clones (Table 1) with the highest cell surface binding activities were selected for production and purification of mAb for further in vitro and in vivo
5 characterization. One hybridoma (Table 1) with no cell surface binding activity was also selected for production and purification of mAb as the control mAb in future functional studies.

The effect of 1025 inhibition on tumorigenesis and tumor growth was determined by treating the human breast tumor cell line MDA-MB-435 with either agent and inoculating
10 treated cells into nude mice. The tumors formed from treated cells showed substantial inhibition in growth rate compared with cells treated by negative controls (Figure 57) and confirmed the 1025 inhibition effect on cells in culture also applies to tumorigenesis and tumor growth.

Other embodiments and uses of the invention will be apparent to those skilled in the
15 art from consideration of the specification and practice of the invention disclosed herein. All references and materials cited herein, including all U.S. and foreign patents and patent applications, are specifically and entirely hereby incorporated herein by reference. It is intended that the specification and examples be considered exemplary only, with the true scope and spirit of the invention indicated by the following claims

Table 1

Search query: TGGCCAATAA (SEQ ID NO:36)

Color Code										
Tags per 200,000	<2	<4	<8	<16	<32	<64	<128	<256	<512	>512
I. Library										
Total Tags in Library										
Tags per 200,000										
Color Code										
SAGE_White_Blood_Cells_normal_AP	31985	43								
SAGE_Breast_carcinoma_metastasis_B_2	49794	40								
SAGE_Breast_metastatic_carcinoma_B_95-260	45087	35								
SAGE_Breast_carcinoma_CL_ZR75_1_tamoxifen	40052	34								
SAGE_Breast_carcinoma_myoepithelium_AP_DCIS7	37435	32								
SAGE_Breast_carcinoma_AP_DCIS-2	28719	27								
SAGE_Breast_carcinoma_B_IDC-5	60451	26								
SAGE_Breast_carcinoma_CL_ZR75_1_estrogen	38797	25								
SAGE_Placenta_normal_B_1	118083	23								
SAGE_Breast_carcinoma_B_95-348	60343	19								
SAGE_Breast_carcinoma_B_DCIS-4	60605	19								
SAGE_Stomach_cancer_B_G189	63075	19								
SAGE_Pancreas_normal_CS_HX	31985	18								
SAGE_Peritoneum_normal_B_13	53527	18								
SAGE_Prostate_adenocarcinoma_MD_PR317	64951	18								
SAGE_Prostate_carcinoma_CL_LNCaP-T	43542	18								
SAGE_Brain_glioblastoma_CL_H54+LacZ	66908	17								
SAGE_Breast_carcinoma_myoepithelium_AP_DCIS6	81452	17								
SAGE_Vascular_normal_CS_VEGF+	57316	17								
SAGE_Breast_carcinoma_CL_MCF7estradiol_10H	59583	16								
SAGE_Breast_carcinoma_CL_MCF7estradiol_3h	59583	16								
SAGE_Brain_astrocytoma_grade_II_B_H563	88568	15								
SAGE_Breast_carcinoma_B_95-259	39364	15								
SAGE_Universal_reference_human_RNA_CL	51729	15								
SAGE_Breast_carcinoma_MD_DCIS	40783	14								
SAGE_Ovary_adenocarcinoma_B_OVT-6	41443	14								
SAGE_Brain_astrocytoma_grade_II_B_H388	106285	13								

1. Table 2 -Virtual Northern

Expression Pattern for cluster Hs.57988Text Legend

Tissue	EST Data		SAGE Data		EST Data		SAGE Data		P
	Normal	Cancer	Normal	Cancer	Normal	Cancer	Normal	Cancer	
ALL TISSUES			46 / 1989425	77 / 2012352	0.00	196 / 4801392	78 / 2516172	196 / 4801392	0.02
brain			6 / 224322	3 / 146887	0.38	45 / 1697717	2 / 309734	45 / 1697717	0.02
cerebellum			0 / 4079	0 / 0	--	5 / 252749	1 / 90885	5 / 252749	0.35
cervix			0 / 1052	0 / 41849	--	--	--	--	--
colon			0 / 17509	17 / 145347	0.14	4 / 325836	4 / 98089	4 / 325836	0.13
eye			2 / 56429	0 / 45229	0.28	--	--	--	--
heart		--	2 / 57248	--	--	--	3 / 83063	--	--
kidney			3 / 63353	2 / 75055	0.34	0 /	2 / 106467	0 /	--
liver			0 / 53158	2 / 72873	0.29	0 /	0 / 66308	0 /	--
lung			4 / 103278	4 / 161191	0.33	--	2 / 88708	--	--

lymph node				--	0 / 77166	0 / 48390	--	0 /	--	--
mammary gland					1 / 41075	3 / 88612	0.43	13 / 375224	100 / 1256825	0.00
ovary					0 / 8152	4 / 83675	0.39	3 / 94887	5 / 179472	0.46
pancreas					0 / 7614	3 / 61447	0.40	5 / 64208	5 / 189999	0.11
pineal gland				--	0 / 6287	2 / 8491	0.28	--	--	--
placenta				--	7 / 190882	2 / 40610	0.43	16 / 207348	--	--
pooled tissue				--	6 / 303542	2 / 28022	0.22	--	--	--
prostate					0 / 62862	6 / 53816	0.04	7 / 266949	15 / 491794	0.38

Table III.**Transmembrane analysis based on SOSUI search:**

Query title : ICT-1 24

Total length : 855 A. A.

Average of hydrophobicity : -0.269240

**2. This amino acid sequence is of a MEMBRANE PROTEIN
which have 6 transmembrane helices.**

No.	N terminal	transmembrane region	C terminal	type	length
1	409	WLTFVHSLVTILAVCIYGIAPVG	431	PRIMARY	23
2	656	LWLSLFLHAGILHCLVSICFQMT	678	PRIMARY	23
3	698	LSGVTGNLASAIFLPYRAEVGPA	720	SECONDARY	23
4	745	WRAFFKLLAVVFLFTFGLLPWI	767	PRIMARY	23
5	773	ISGFISGLFLSFAFLPYISFGK	794	SECONDARY	22
6	803	QIIFQVVFLGLLAGLVVLFYVY	825	PRIMARY	23

Table 4. Hybridoma clones selected for production and purification of mAb based on cell surface binding activities

Name of hybridoma	ICT1025 protein binding activity	Cell surface binding activity in MDA-MB-435 cells	Cell surface binding activity in HT29 cells
ICT1025-4G4	Yes	Very high (1.5)	Very high (1.3)
ICT1025-1A7	Yes	High (1.1)	High (0.8)
ICT1025-3C9	Yes	High (1.1)	Medium (0.6)
ICT1025-1D9	Yes	High (0.9)	Medium (0.5)
ICT1025-5F9	Yes	High (1.0)	High (1.0)
ICT1025-5E8	Yes	High (0.9)	Medium (0.6)
ICT1025-4H3	Yes	No (<0.1)	No (<0.1)

What is claimed is:

1. A method for treating a disease in a mammal associated with undesirable activity or expression of ICT1030 peptide, comprising applying a composition that interacts with the ICT1030 peptide or gene, wherein the composition is capable of enhancing expression or activity of the ICT1030 when introduced into a tissue of the mammal.
2. The method according to claim 1, wherein the disease is cancer or a precancerous growth.
3. The method according to claim 1, wherein the tissue is a breast tissue, a colon tissue, a prostate tissue, a skin tissue, a bone tissue, a parotid gland tissue, a pancreatic tissue, a kidney tissue, a uterine cervix tissue, a lymph node tissue, or an ovarian tissue.
4. The method according to claim 1 wherein the composition is a nucleic acid.
5. The method according to claim 4, wherein the nucleic acid is a decoy molecule, a decoy DNA, a double stranded DNA, a single-stranded DNA, a complexed DNA, an encapsulated DNA, a viral DNA, a plasmid DNA, a naked RNA, an encapsulated RNA, a viral RNA, a double stranded RNA, a molecule capable of enhancing expression of the target ICTE1030 gene, or combinations thereof.
6. The method according to claim 4, wherein the nucleic acid molecule is substantially double stranded and has a length of about one hundred base pairs or less.
7. The method according to claim 4, wherein the nucleic acid composition comprises a siRNA, an RNAi or an shRNA or a nucleic acid molecule capable of encoding a siRNA, an RNAi or an shRNA.
8. The method according to claim 4, wherein the nucleic acid composition is a nucleic acid molecule capable of encoding a siRNA, an RNAi or an shRNA, and wherein the nucleic acid molecule is a plasmid, cosmid, bacteriophage, or viral vector.
9. The method according to claim 8, wherein the vector is a retroviral or adenoviral vector.
10. The method according to claim 4, wherein the nucleic acid composition comprises at least one molecule selected from the group consisting of an siRNA, an RNAi, and an shRNA, and wherein the molecule enhances expression of the target ICTE1030 gene in the mammal.
11. The method according to claim 1, wherein the mammal is a human.
12. The method according to claim 4, wherein the nucleic acid forms a triple helix with the target ICTE1030 gene-encoding nucleic acid.

13. The method of claim 1, wherein the target ICT1030 gene comprises a polynucleotide selected from the group consisting of:
 - (a) a polynucleotide encoding the polypeptide set forth in SEQ ID NO:2;
 - (b) a polynucleotide set forth in SEQ ID NO:1 or SEQ ID NO:3; and
 - (c) a polynucleotide having at least about 90% sequence identity to the polynucleotide of (a) or (b).
14. The method of claim 1, wherein the target ICT1030 gene comprises a polynucleotide having at least about 90% sequence identity to SEQ ID NO:1 or SEQ ID NO:3.
15. The method of claim 1, wherein the target ICT1030 gene comprises a polynucleotide having at least about 90% sequence identity to a polynucleotide encoding the polypeptide as set forth in SEQ ID NO:2.
16. The method of claim 1, wherein the target ICT1030 gene comprises a polynucleotide having at least about 95% sequence identity to a polynucleotide encoding the polypeptide as set forth in SEQ ID NO:2.
17. The method of claim 1, wherein the target ICT1030 gene comprises a polynucleotide having at least about 95% sequence identity to a polynucleotide encoding the polypeptide set forth in SEQ ID NO:2.
18. The method according to claim 13, wherein the nucleic acid molecule is substantially double stranded and has a length of about one hundred base pairs or less.
19. The method according to claim 13, wherein the nucleic acid composition comprises a siRNA, an RNAi or an shRNA or a nucleic acid molecule capable of encoding a siRNA, an RNAi or an shRNA.
20. The method according to claim 19, wherein the nucleic acid composition is a nucleic acid molecule capable of encoding a siRNA, an RNAi or an shRNA, and wherein the nucleic acid molecule is a plasmid, cosmid, bacteriophage, or viral vector.
21. The method according to claim 20, wherein the vector is a retroviral or adenoviral vector.
22. A method of administering nucleic acid to a patient in need thereof, wherein the nucleic acid molecule is delivered in the form of a naked oligonucleotide or a vector, wherein the nucleic acid interacts with the target ICT1030 gene.
23. The method of claim 22, wherein the nucleic acid is delivered as a vector, wherein the vector is a plasmid, cosmid, bacteriophage, or a virus.

24. The method of claim 23, wherein the vector is a retrovirus or an adenovirus based vector.
25. The method of claim 22, wherein the nucleic acid enhances the target ICT1030 gene expression in a mammalian cell.
26. The method of claim 22, wherein the cell is a human cell.
27. The method of claim 1 wherein the composition is the ICT1030 polypeptide set forth in SEQ ID NO:2 or a polypeptide having at least 90% sequence identity to the polypeptide of SEQ ID NO:2 and the biological activity of the ICT 1030 polypeptide.
28. The method of claim 1 wherein the composition is an antibody specific for the ICT1030 polypeptide set forth in SEQ ID NO:2 or a polypeptide having having at least 90% sequence identity to the polypeptide of SEQ ID NO:2.
29. A method for inhibiting cancer or precancerous growth in a mammalian tissue, comprising contacting the tissue with an enhancer that interacts with the target ICT1030 and thereby enhances the target ICT1030 expression or activity.
30. The method according to claim 29, wherein the tissue is a breast tissue, colon tissue, a prostate tissue, a skin tissue, a bone tissue, a parotid gland tissue, a pancreatic tissue, a kidney tissue, a uterine cervix tissue, a lymph node tissue, or an ovarian tissue.
31. The method according to claim 29, wherein the composition comprises a nucleic acid selected from the group consisting of an siRNA, an RNAi, and an shRNA, and wherein the molecule enhances expression of the target ICT1030 gene in the mammal.
32. The method according to claim 29, wherein the mammal is a human.
33. A method for treating a disease in a mammal associated with undesirable expression or activity of ICT1031, ICT1024, ICT 1025, or ICT1003 peptide, comprising applying a composition containing an inhibitor that interacts with the ICT1031 ICT1024, ICT 1025, or ICT1003 peptide or DNA or RNA, wherein the composition is capable of reducing expression or activity of the ICT1031, ICT1024, ICT 1025, or ICT1003 peptide when introduced into a tissue of the mammal.
34. The method according to claim 33, wherein the disease is cancer or a precancerous growth.
35. The method according to claim 33, wherein the tissue is a breast tissue, a colon tissue, a prostate tissue, a skin tissue, a bone tissue, a parotid gland tissue, a pancreatic tissue, a kidney tissue, a uterine cervix tissue, a lymph node tissue, or an ovarian tissue.
36. The method according to claim 33, wherein the composition is nucleic acid.

37. The method according to claim 36, wherein the inhibitor is a siRNA, an RNAi, a shRNA, an antisense RNA, an antisense DNA, a decoy molecule, a decoy DNA, a double stranded DNA, a single-stranded DNA, a complexed DNA, an encapsulated DNA, a viral DNA, a plasmid DNA, a naked RNA, an encapsulated RNA, a viral RNA, a double stranded RNA, a molecule capable of generating RNA interference, or combinations thereof.
38. The method according to claim 36, wherein the nucleic acid molecule is substantially double stranded and has a length of about one hundred base pairs or less.
39. The method according to claim 38, wherein the nucleic acid composition comprises a siRNA, an RNAi or an shRNA or a nucleic acid molecule capable of encoding a siRNA, an RNAi or an shRNA.
40. The method according to claim 39, wherein the nucleic acid composition is a nucleic acid molecule capable of encoding a siRNA, an RNAi or an shRNA, and wherein the nucleic acid molecule is a plasmid, cosmid, bacteriophage, or viral vector.
41. The method according to claim 40, wherein the vector is a retroviral or adenoviral vector.
42. The method according to claim 36, wherein the nucleic acid composition comprises at least one molecule selected from the group consisting of an siRNA, an RNAi, and an shRNA, and wherein the molecule causes post-transcriptional silencing of the target ICT1031, ICT1024, ICT 1025, or ICT1003 gene in the mammal.
43. The method according to claim 33, wherein the mammal is a human.
44. The method according to claim 36, wherein the inhibitor forms a triple helix with a target ICT1031-encoding nucleic acid.
45. The method of claim 33, wherein the target ICT1031 gene comprises a polynucleotide selected from the group consisting of:
- (a) a polynucleotide encoding the polypeptide set forth in SEQ ID NO:5;
 - (b) a polynucleotide set forth in SEQ ID NO:4; and
 - (c) a polynucleotide having at least about 90% sequence identity to the polynucleotide of a) or b).
46. The method of claim 45, wherein the target ICT1031 gene comprises a polynucleotide having at least about 95% sequence identity to a polynucleotide encoding the polypeptide as set forth in SEQ ID NO:5.
47. The method of claim 45, wherein the target ICT1031 gene comprises a polynucleotide having at least about 95% sequence identity to a polynucleotide set forth in SEQ ID NO:4.

48. The method of claim 33, wherein the target ICT1003 gene comprises a polynucleotide selected from the group consisting of:

- (a) a polynucleotide encoding the polypeptide set forth in SEQ ID NO:7;
- (b) a polynucleotide set forth in SEQ ID NO:6 or SEQ ID NO:8; and
- (c) a polynucleotide having at least about 90% sequence identity to the polynucleotide of a) or b).

49. The method of claim 48, wherein the target ICT1003 gene comprises a polynucleotide having at least about 95% sequence identity to a polynucleotide encoding the polypeptide as set forth in SEQ ID NO:7.

50. The method of claim 48, wherein the target ICT1003 gene comprises a polynucleotide having at least about 95% sequence identity to a polynucleotide set forth in SEQ ID NO:8.

51. The method of claim 33, wherein the target ICT1024 gene comprises a polynucleotide selected from the group consisting of:

- (a) a polynucleotide encoding the polypeptide set forth in SEQ ID NO:37;
- (b) a polynucleotide set forth in SEQ ID NOs: 58, 60, 61, 62, 64, 66, 68 or 69; and
- (c) a polynucleotide having at least about 90% sequence identity to the polynucleotide of a) or b).

52. The method of claim 51, wherein the target ICT1024 gene comprises a polynucleotide having at least about 95% sequence identity to a polynucleotide encoding the polypeptide as set forth in SEQ ID NO:37.

53. The method of claim 51, wherein the target ICT1024 gene comprises a polynucleotide having at least about 95% sequence identity to a polynucleotide set forth in SEQ ID NOs. 58, 60, 61, 62, 64, 66, 68 or 69.

54. The method of claim 33, wherein the target ICT1025 gene comprises a polynucleotide selected from the group consisting of:

- (a) a polynucleotide encoding the polypeptide set forth in SEQ ID NO:71;
- (b) a polynucleotide set forth in SEQ ID NO: 70; and
- (c) a polynucleotide having at least about 90% sequence identity to the polynucleotide of a) or b).

55. The method of claim 54, wherein the target ICT1025 gene comprises a polynucleotide having at least about 95% sequence identity to a polynucleotide encoding the polypeptide as set forth in SEQ ID NO:70.

56. The method of claim 54, wherein the target ICT1025 gene comprises a polynucleotide having at least about 95% sequence identity to a polynucleotide set forth in SEQ ID NO:71.
57. A method for inhibiting cancer or precancerous growth in a mammalian tissue, comprising contacting the tissue with an inhibitor that interacts with a target ICT1031, ICT1024, ICT 1025, or ICT1003 DNA or RNA and thereby reduces target ICT1031, ICT1024, ICT 1025, or ICT1003 gene expression.
58. The method according to claim 57, wherein the tissue is a breast tissue, colon tissue, a prostate tissue, a skin tissue, a bone tissue, a parotid gland tissue, a pancreatic tissue, a kidney tissue, a uterine cervix tissue, a lymph node tissue, or an ovarian tissue.
59. The method according to claim 57, wherein the inhibitor is a siRNA, an RNAi, a shRNA, an antisense RNA, an antisense DNA, a decoy molecule, a decoy DNA, a double stranded DNA, a single-stranded DNA, a complexed DNA, an encapsulated DNA, a viral DNA, a plasmid DNA, a naked RNA, an encapsulated RNA, a viral RNA, a double stranded RNA, a molecule capable of generating RNA interference, or combinations thereof.
60. The method according to claim 57, wherein the nucleic acid molecule is substantially double stranded and has a length of about one hundred base pairs or less.
61. The method according to claim 57, wherein the nucleic acid composition comprises a siRNA, an RNAi or an shRNA or a nucleic acid molecule capable of encoding a siRNA, an RNAi or an shRNA.
62. The method according to claim 57, wherein the nucleic acid composition is a nucleic acid molecule capable of encoding a siRNA, an RNAi or an shRNA, and wherein the nucleic acid molecule is a plasmid, cosmid, bacteriophage, or viral vector.
63. The method according to claim 62, wherein the vector is a retroviral or adenoviral vector.
64. The method according to claim 57, wherein the nucleic acid composition comprises at least one molecule selected from the group consisting of an siRNA, an RNAi, and an shRNA, and wherein the molecule causes post-transcriptional silencing of the target ICT1031, ICT1024, ICT 1025, or ICT1003 gene in the mammal.
65. The method according to claim 57, wherein the mammal is a human.
66. The method according to claim 57, wherein the inhibitor forms a triple helix with a target ICT1031, ICT1024, ICT 1025, or ICT1003-encoding nucleic acid.
67. The method of administering siRNA to a patient in need thereof, wherein the siRNA molecule is delivered in the form of a naked oligonucleotide or a vector, wherein the

siRNA interacts with a target ICT1031, ICT1024, ICT 1025, or ICT1003 gene or a target ICT1031, ICT1024, ICT 1025, or ICT1003 mRNA transcript.

68. The method of claim 67, wherein the siRNA is delivered as a vector, wherein the vector is a plasmid, cosmid, bacteriophage, or a virus.

69. The method of claim 68, wherein the vector is a retrovirus or an adenovirus based vector.

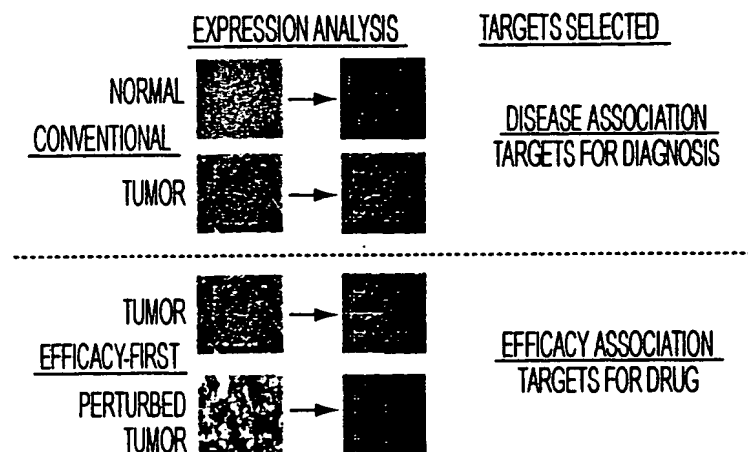
70. A method of blocking in vivo expression of a target ICT1031, ICT1024, ICT 1025, or ICT1003 gene by administering a vector to a patient in need thereof, wherein the vector containing a target ICT1031, ICT1024, ICT 1025, or ICT1003 siRNA.

71. The method of claim 70, wherein the siRNA interferes with target ICT1031, ICT1024, ICT 1025, or ICT1003 gene expression.

72. The method of claim 71, wherein the siRNA causes post-transcriptional silencing of the target ICT1031, ICT1024, ICT 1025, or ICT1003 gene in a mammalian cell.

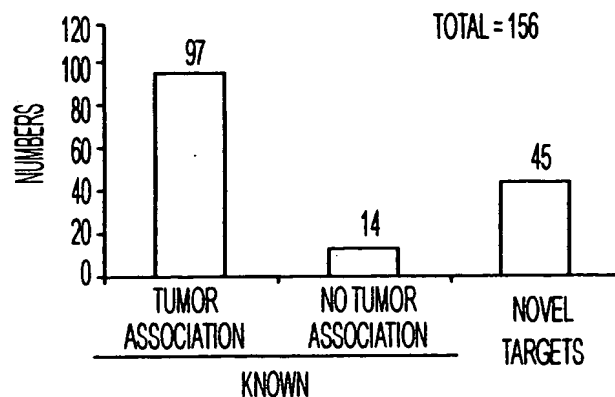
73. The method of claim 72, wherein the cell is a human cell.

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ADVANTAGE OF EFFICACY-FIRST DISCOVERY™ METHOD

FIG. 1



HIGHLY ENRICHED TUMOR TARGETS

FIG. 2

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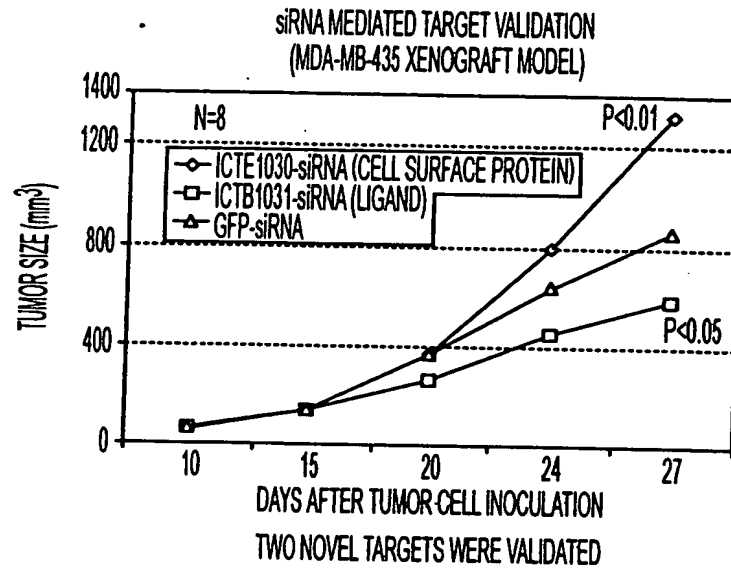


FIG. 3

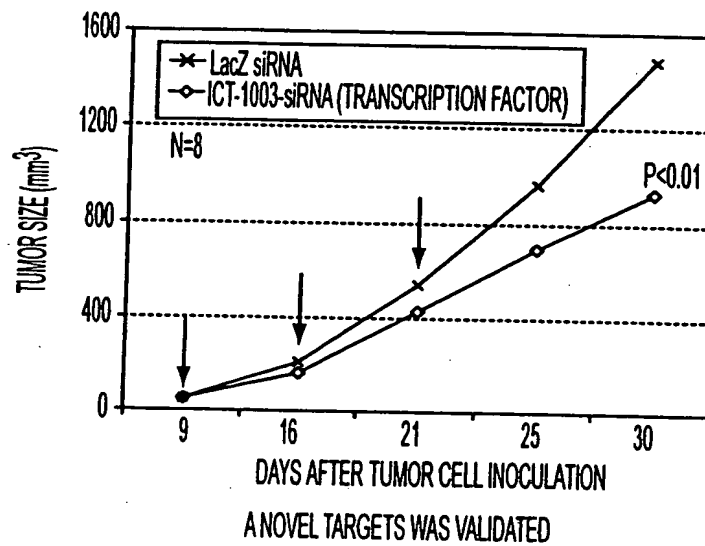


FIG. 4

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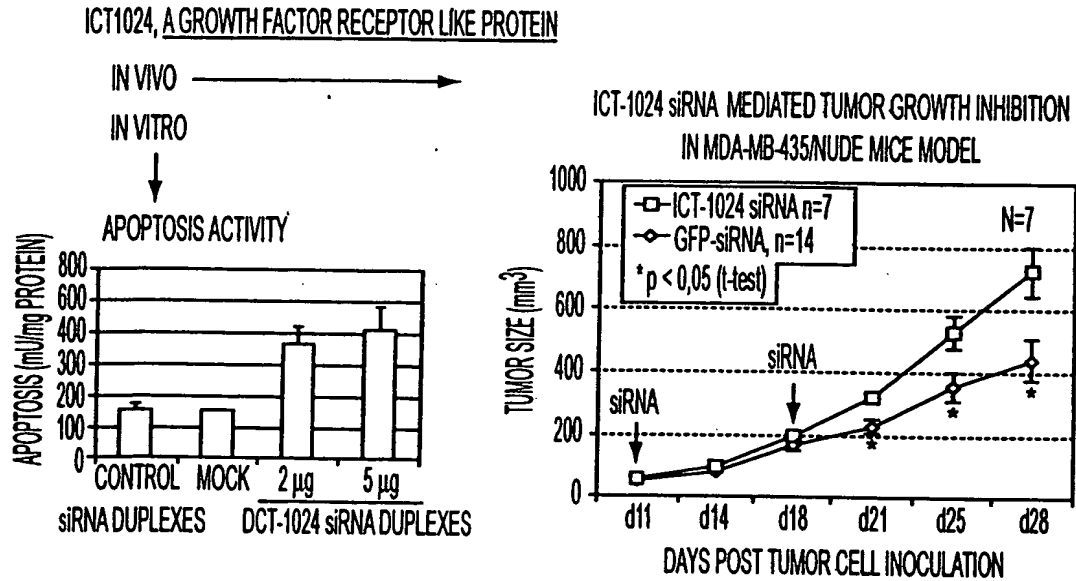


FIG. 5

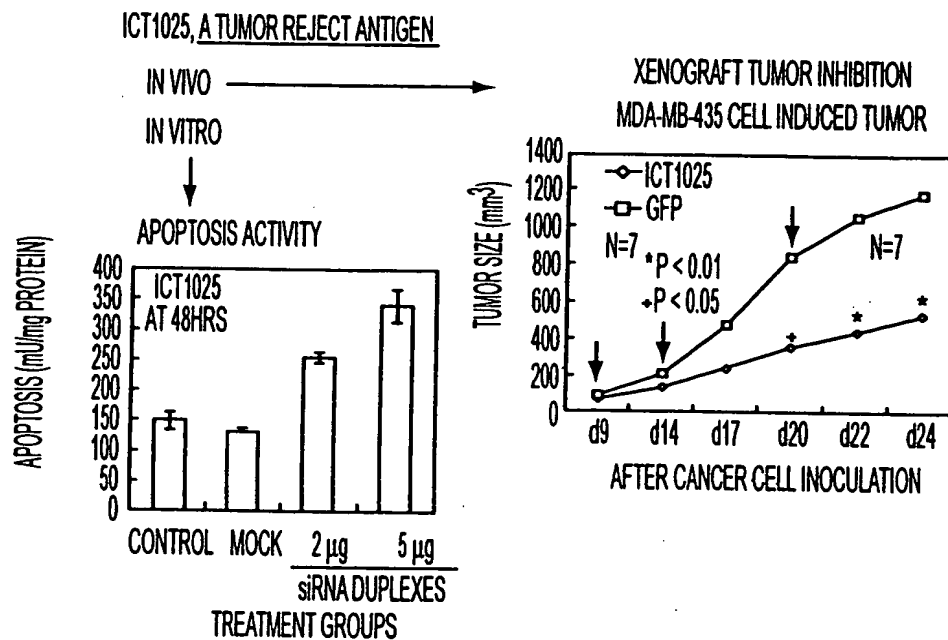


FIG. 6

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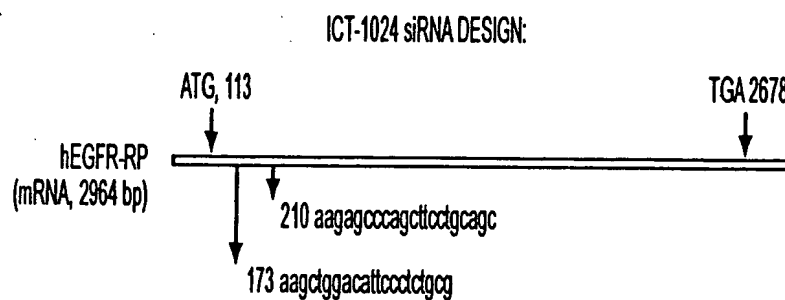


FIG. 7

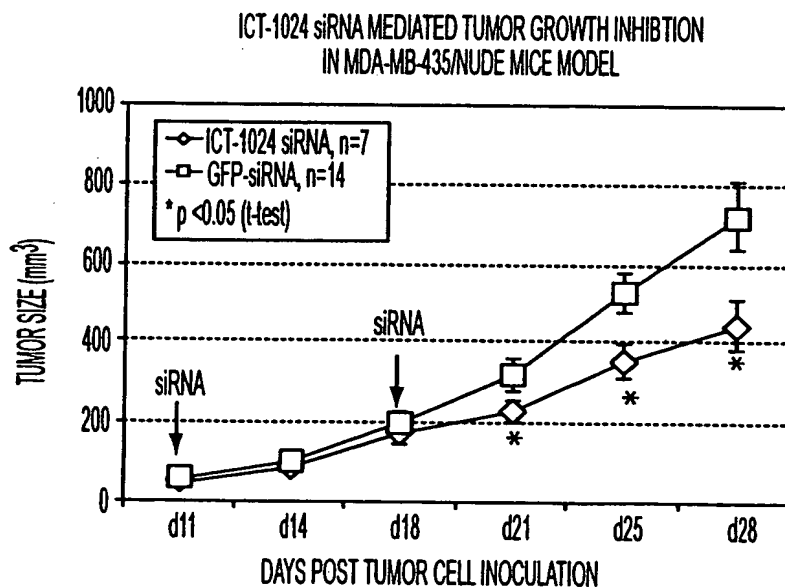


FIG. 8

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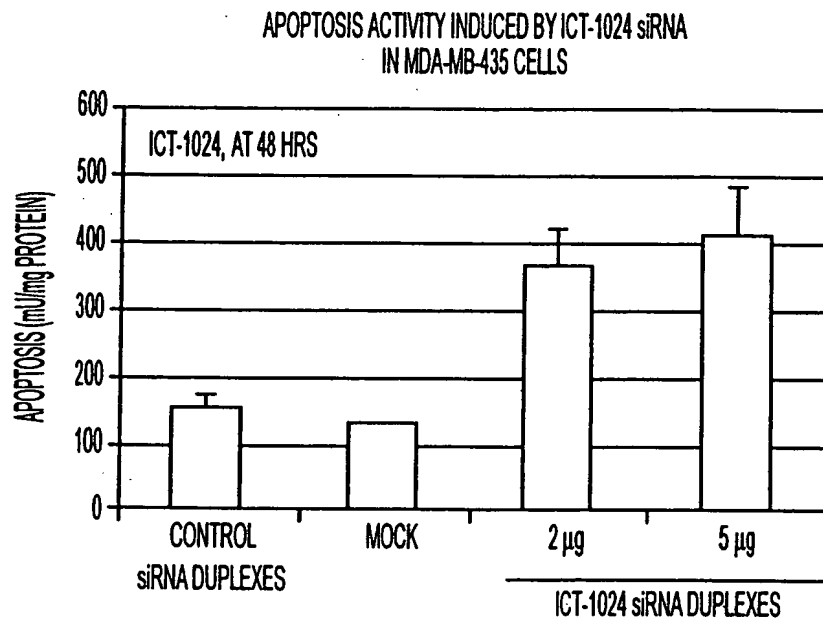


FIG. 9

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SAGE/MICROARRAY DATA

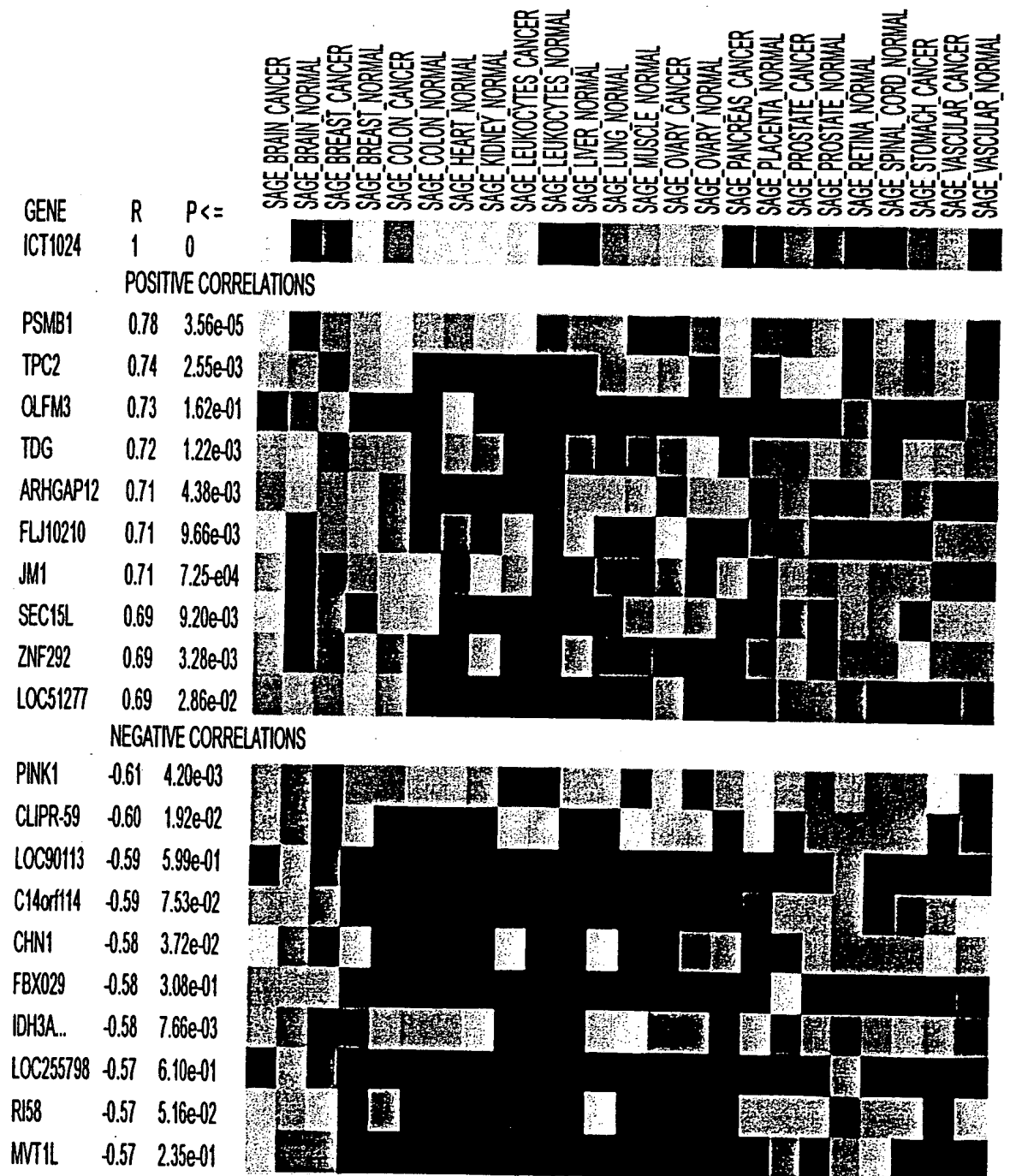


FIG. 10

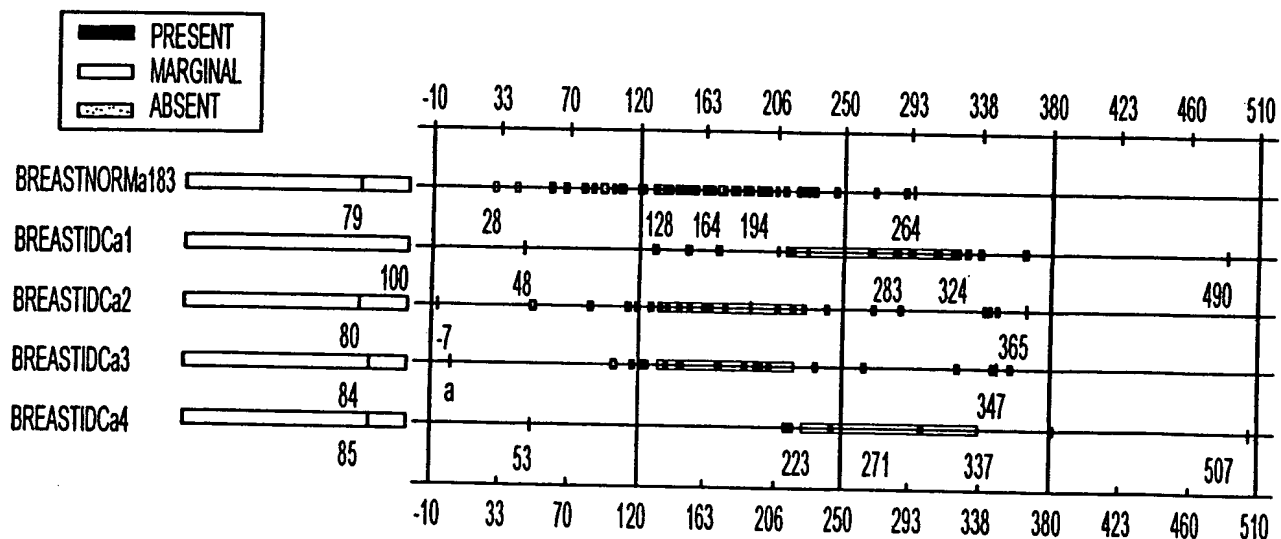
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CANCER TISSUE DISTRIBUTION

FRAGMENT NAME	218686 s at			
SEQ. ACCESSION	NM 022450			
GENE NAME	LIKELY ORTHOLOG OF MOUSE EPIDERMAL GROWTH FACTOR RECEPTOR, RELATED SEQUENCE			

BREAST NORMAL VS. INFILTRATING DUCT CARCINOMA (IDC) STAGE I	
FOLD CHANGE	1.69
DIRECTION	UP
FOLD CHANGE P-VALUE	0

	NORMAL	IDC S-I	IDC S-II	IDC S-III	IDC S-IV
# OF SAMPLES (n)	83	19	30	19	6
MEAN	159.73	264.5	192.96	195.16	284.29
STDDEV	50.03	70.64	82.37	77.41	71.09
MEDIAN	164.53	283.05	191.27	187.7	271.59
PRESENT CAL%	79	100	80	84	83



ICT1024 IS HIGHLY UP REGULATED IN ALL STAGE I BREAST TUMOR SAMPLES (100%)

FIG. 11

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Consensus in rhomboid family

		10	20	30	40	50	60
	******
Human	consensus 1	PLQPGQLWR	--LITSMFLHAGILHLLFNMLSLLFFGIPLERRIGSVRFLLLYLLSGLAG-	57			
Yeast	gi 9963865 99	ALRNWQVYR	--LVTYIFVYENPISLLCGAIIWRPAGNFERTVGTVRHCFFTVIFAIFS-	155			
Bacteria	gi 3738201 47	LLQKRQLYE	--IITYVTLHLSMLHIVFNFVSLLPAMSQFEKKQGTACILVTVIPYTLFp	104			
Yeast	gi 1653749 49	PRSLEGLRG	--IVFAPFLHADFGHLIANSVPFVVLAWLVMLQ-EVSDFWIVTIIITMVVG-	104			
Human	gi 13621505 60	sksnarpvvaigds	DIYSYRIWS-FFCQWINTIFCWSNRRRLGLTPFLLLYVLSGVMG-	117			
Bacteria	gi 20139804 103	PEKREEAWR	--FISYMLVHagVQHILGNLCMQLVLGIPLEMVHKGLRVGLVYLAGVIAG-	159			
Human	gi 1169951 129	PTLKFEFWR	--YFTHALMHFSLMHILFNLLWWYLGGAVEKRLGSGKLIVIRSISALLS-	185			
Bacteria	gi 11066250 198	SNPASKVLCsp	MLLSTFSHPSLFHMAANMYVLWSFSSSIVNILQEQFMAYVLSAGVIS-	256			
Bacteria	gi 13813618 68	yLVIKGYyse	-LFTSIFITNSFVDFIFNFISLYVIYLIFGSRAGKHEYGIFILAGILGN-	125			
Plant	gi 9294149 242	IFKHKDLKR	--LFLSAFYHVNPHLVNMMSLWKGIKLETSMGSSSEFASMVFTLIGMS-	298			
		70	80	90	100	110	120
	******
Human	consensus 58	SLLSLLSPAS	----TPSVGASGAIFGLLGALLVLLPLNRILLNP--GAALFLLLGII	110			
Yeast	gi 9963865 156	AIIFLSFEAVSs	----LSKLGEVEDARGFTPVAFAMLGVTTVRSRMraLVFGMVVPSVL	211			
Bacteria	gi 3738201 105	GIMHLIVYHFFlr	kyVSIAGLSGWAFAFISASCVHSPQRLISFFN-----LFSIPAYCF	159			
Yeast	gi 1653749 105	GLGVWLIAPPN	----TVTVGASILIFGYLGFLFRGWFPQKNLASIV1-SIVVLVLYGSA	158			
Human	gi 13621505 118	NAFTFWLTPETv	-----AAGASTSLFGLFAAIVVLSFLGKQKALKD1-GKSYQTLIV-V	169			
Bacteria	gi 20139804 160	SLASSIFDPLR	-----YLVGASGGVYALMGGYFMNVLVNFQEMIPA--FGIFRLLIIIL	211			
Human	gi 1169951 186	GYVQQKFSGPW	-----FGGLSGVVYALMGYVWLGRGERDPQSG-----IYLQRLGI	230			
Bacteria	gi 11066250 257	NFVSYLGKVATgr	--yGPSLGASGAIMTVLAAVCTKIPEGR-LA-----IIFLPMFTF	306			
Bacteria	gi 13813618 126	LLTVIFYSPFT	-----LSSGASGGIFGLLSYTYFYDFLKKDNLG----VYGLVFLVSVF	175			
Plant	gi 9294149 299	QGVTLALLAKSL	-----LLLFDYDRAYYNEYAVGFSGVLFAMKVVLNSq-AEDYSSVYGIL	352			
		130	140	150	160	170	
	*****	
Human	consensus 111	LLNLLLGL	--LPGISNFG-----HLGGLLAGLLGFLLLRRPR	146			
Yeast	gi 9963865 212	VPWLLLGAswl	IPQTSFLS-----NVCGLSIGLAYAHLFFFHRP	250			
Bacteria	gi 3738201 160	PIIYLINTtil	VPKASFIG-----HASGAVMGYCTPFMLGSIPL	198			
Yeast	gi 1653749 159	LWGLLPGR	---AGVSWQG-----HLFGFIGGAIAAWLIAREKH	193			
Human	gi 13621505 170	NLLMNLF	---MPNVSMAG-----HIGGVVGALLSIVFPTKMR	204			
Bacteria	gi 20139804 212	IIVLDMGF	---ALYRRFFVpedgsp-vsfaaHIAGGFAGMSIGYTVFSCFD	258			
Human	gi 1169951 231	IFALIWIW	---AGWFDLFGmsma-----ngaHIAGLAVGLAMAFVDSLNR	273			
Bacteria	gi 13813618 176	TAGNALKA	---IIAMDTAGmilgwkfddhaaHLGGALFGIWIYVYGHელიW	354			
Plant	gi 9294149 353	GVSDLIFP	---NVNVVA-----HIGGILGGIMYAVVYYLIRS	209			
		VPTKYAAWa	eLILVQMFVpnas-----flgHLGGILAGIYYLKLKGSYSG	397			

FIG. 12

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Human rhomboid Proteins Human Rhomboid Family Protein Alignments

ICT-1024	1	msearrdstsslqrktpwklkipsavpltae--eps-----flqlrrqalrsvmpaetahisphhrlrrpvlqrqtsaitqtrrgtdawfgvskdadstqkwrksirhsqry
HRhomboid 2	1	-----
HRhomboid 3	1	-----
HRhomboid 4	1	mgeh-----psppavaacaeae-----rieeleneaeerlpaaped-----hwkv-----lfdqfdpntgyi-----stgkfrslleshsk--
HRhomboid 5	1	-----
HRhomboid 6	1	mqrtsrgintglilllsqifhvginnippvtla--tlalniwfflnp-----qkplyssclsvekyqg-----kd-----wqr-----wagrg-wgcggaw
ICT-1024	114	gklktpvlrelclpsqdvnslstetppplyvgpcqlgmqkllpdlargrafrvaddtaeglsaphtpvtpgaaalscfssrsrgfhrlprrrkresvakmsfraaaalmkgrsvrdgtf
HRhomboid 2	1	-----
HRhomboid 3	1	-----
HRhomboid 4	70	-----ldphkrevllaladshadg-----msvahmslqaaaaalkqrsvidatg-----mlnmngremkee--
HRhomboid 5	18	g-----asvgrsceeltavltppqll-----grrfnf-----fiqqkcgfrkaprk-----qigyqdfvslmsnk--rsnsf
HRhomboid 6	68	-----lllspkhl-----add-----wh-----lyfnmasmlwkginle-----veprsdpgts
ICT-1024	234	rra--r--rrsftpasfleedttfdpeldtsffaregilheeilstypdevfespsealkdwetapeqadltggaldraeleshlmplergwrkqkegaapqpkvrlrqevvsta
HRhomboid 2	13	-----leee-----ekmred-----gggkdrakskvh-----
HRhomboid 3	26	qrc--rvwksfafpsfleeedvvdadtfdssfsk-----eemsmddvfyespplsa-syfrgiphsa-----spvsgdgvcpl-----keygrapvp--
HRhomboid 4	108	rqailqg--nrrlsskalleekglsqrl-----irhvayetiprel-----drkwydydytccpp-----
HRhomboid 5	71	gea-----yvitafsvltgvvylillqfavaefndep-----dfkrsc--avgfsgvlfalkvlnmhy-----etvfyfypsp-----
HRhomboid 6	97	rrl--g--srwfa-----
ICT-1024	349	gprgqriavpvrkl-farekrpyglgmvgirltnrtyrkridsfvkrqiedmddhrfftywltfvhsylvtilavciygiap-vqfsqhetvdsvlrrngvyenvkyvqgenfwgppsse
HRhomboid 2	36	-----ri-----vskw-mipek-----sgtyletancfpp-----pviislsae-----lavfily-----
HRhomboid 3	109	gprgkriaskvkhafdrkrhryglgvvgmwnrsyrsissvtqqlsfdsrpyfitywltfvhviitllivctygiap-vqfahvttqlvlrnkgyesvskyiqgenfwgppsii
HRhomboid 4	163	-----pwfmi-----tvtillevaff-----lyngv-----
HRhomboid 5	93	-----ypirsl-i-----kpl-----fftvgft-----gcalfgsaa-iw--qyeslks--rvqsyfdgik-----
HRhomboid 6	166	-----lgfpv-----pnr-----facwvel-----valhifspgtsfaghla-----gilvqlmytq-----gplkk

FIG. 13

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ICT-1024
HRhomboid 2
HRhomboid 3
HRhomboid 4
HRhomboid 5
HRhomboid 6
467 alihlgakfepcmrqpqbhsfiraarethsaacvrndrscvqtseecstlawwkwlpshpsap---elaghrqfsgvchqdprvcdpssedpshewpeditkwpictknsagn
78 -----yavw-----kpgkqw-----
228 dlilhlgakfepcmrqpqbhsfiraarethsaacvrndrscvqtseecstlawwkwlpshpsap---elaghrqfsgvchqdprvcdpssedpshewpeditkwpictknsagn
183 -----slggfvlqthpr-----yknslvy-----
137 -----adwldsr--pqkegdf--keinkw-----wnnlsdgqrvtvgiaanylvfclwr-----pslqrtmlry-----sgssgy
213 im-----eacagfsssgyp-----grgyfins-----
ICT-1024
HRhomboid 2
HRhomboid 3
HRhomboid 4
HRhomboid 5
HRhomboid 6
584 htnbpbmdcvitgrpcicgtkgreitsreycdmrgyfheatlcsqvhcmddvcgll--pfi-npevpdqfyrwlslfihagilhcjvsicfcmvtrldleklagwhriaiyillsq
88 -----itldt-----gilesfpiyspekreeawrfisymvlnhagvqhihlgmclmqvlvgplemwhkqlrvqlvyilagv
348 htgflhmdceikgrpcicgtkgreitsreycefmhgyfheatlcsqvhcldkvcgll--pfi-npevpdqfyrwlslfihagvchclsvvfmqtilrdleklagwhriaiyillsq
204 h-----pqlraqwryltyifmhagiehlgnvlgllvgvplemwhgatrigrlyvyagv
196 ftsnp-----askvlcs-----pml-----lstaifshfmaamvylwsfsssvmllgqeqfmavylsag
243 qdyyp-----grp-----dhveeaprmvdytaglseeq-----leralqasl-----wdr-----g
701 vtgnlasaiflp---yraevpagsqgilacilfvelfqg-wqlarpraff--kllawilftfgl-lpw--i-----dnf-----ahisgfisqilfslafal
157 iagslasifdp---rylvagasgyvalmgvfmvln-fqemipafgr--llliillivldmgf-aly--i-----rffvpdgspsvsaahiaqagfag--msiqyt
465 itgnlasaiflp---yraevpagsqgilacilfvelfqg-wqlarpraff--kllawilftfgl-lpw--i-----dnf-----ahisgfisqilfslafal
259 vagslavsvadm---lapwsgsggyvalvsahlanivm-wsqmkqfklr--mavalicmsmeqgr-aww--lrfhpsayppcpshpsiv-----ahlggavagvtilgwwvl
254 visnfsvlgkvatgrygplsagalmvlaavctkipegrlaiflpmftftagnalkailamtagmilgwkkf-----dha-----ahlggavagvtilgwwvl
287 ntrnspp---p---yghlsp-----
788 pyisfgkfdlyrkrcqilifqvflgllagvlwlyvypvrcewcefltcipftdkfckeyeldaqh---
253 vtsfcdkallkdrfwai-----aaylacvlfavf--vfnitfspan-----
552 pyitfgtsdkyrkralilvslafaglaalwlyvypinwpwiehltciftsrfckeyelgdlh---
359 im-----yegrldqslwlvfamyt-vfvlfavf-----wnifavtl-----ldklpppp
345 -yvtygnelwknr-----eplvkiwheirtnpkkggsk-----hrfdsq-----
303 -----emrrqr-----

FIG. 13(continued)

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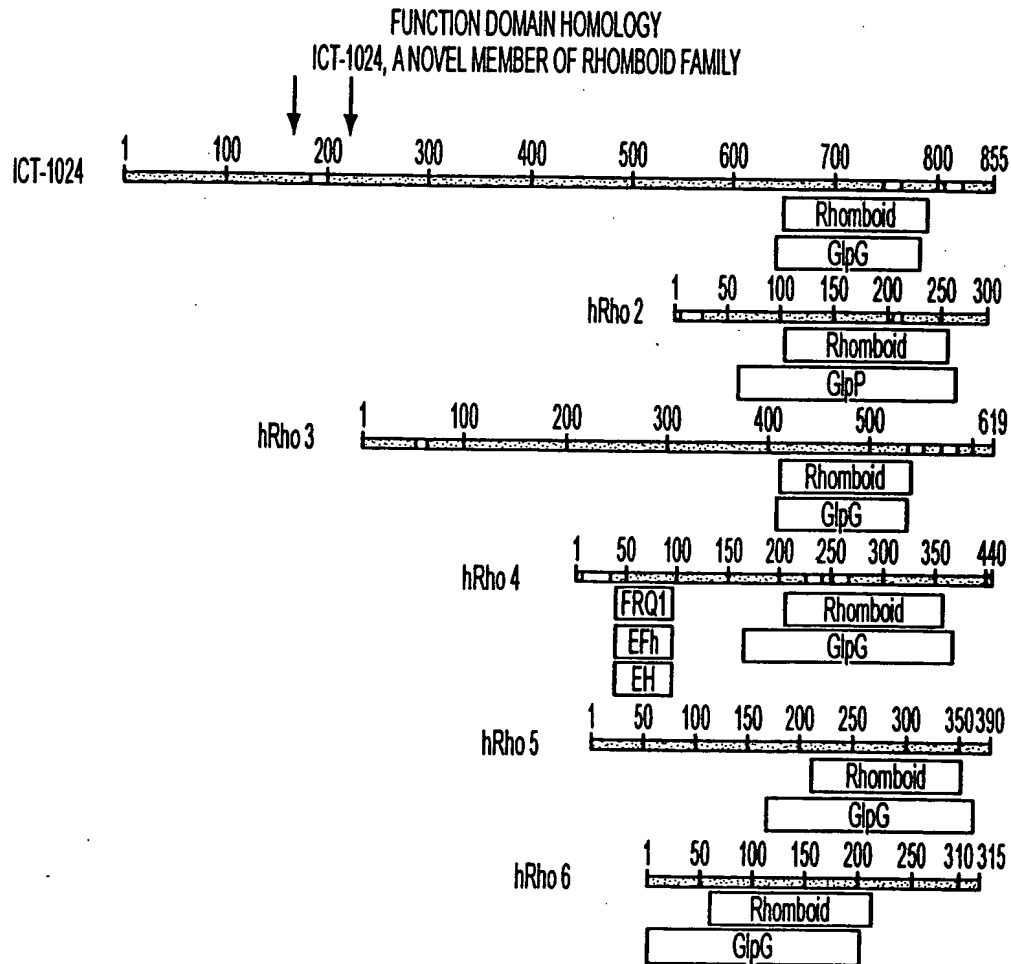


FIG. 14

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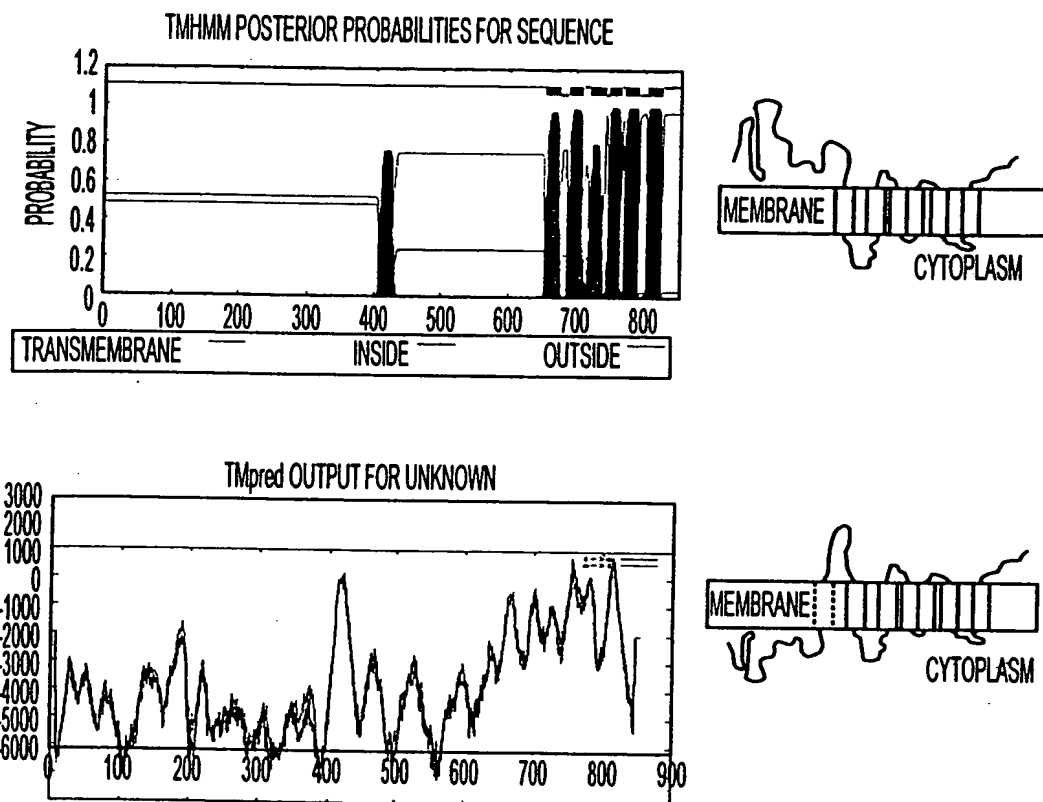


FIG. 15

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ACTIVATION OF EGFRS AND LIGANDS

ICT-1024 INTRAMEMBRANE PROTEASE ACTIVITY

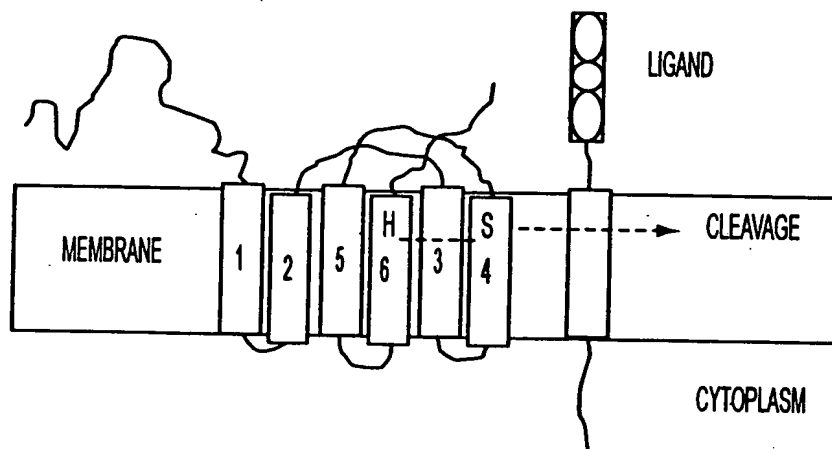


FIG. 16

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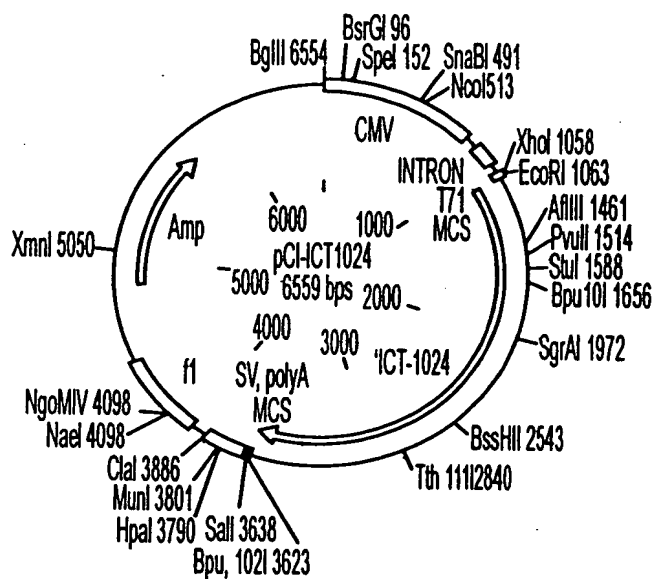


FIG. 17

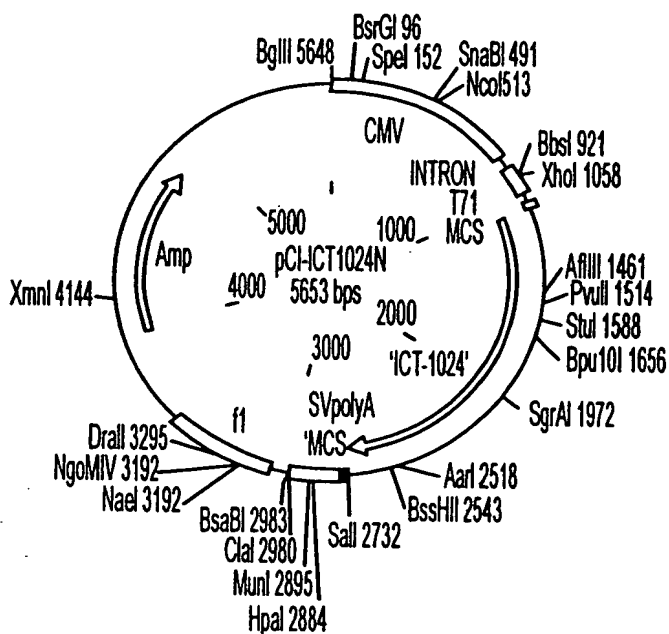


FIG. 18

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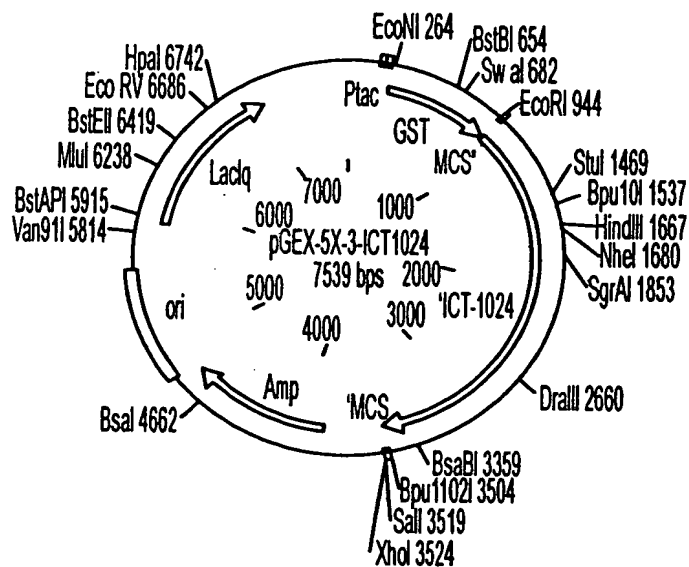


FIG. 19

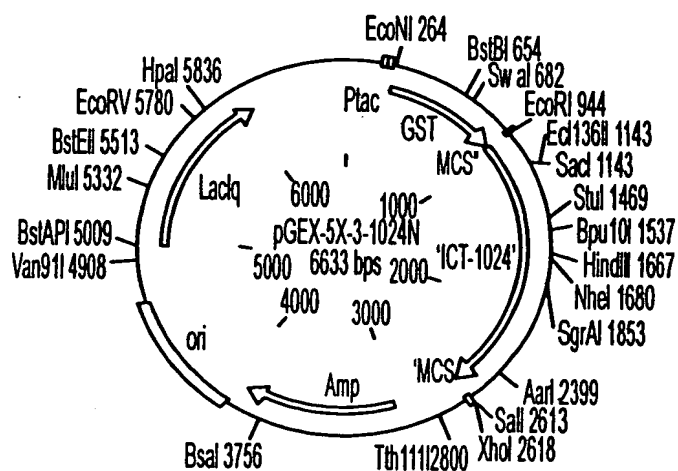
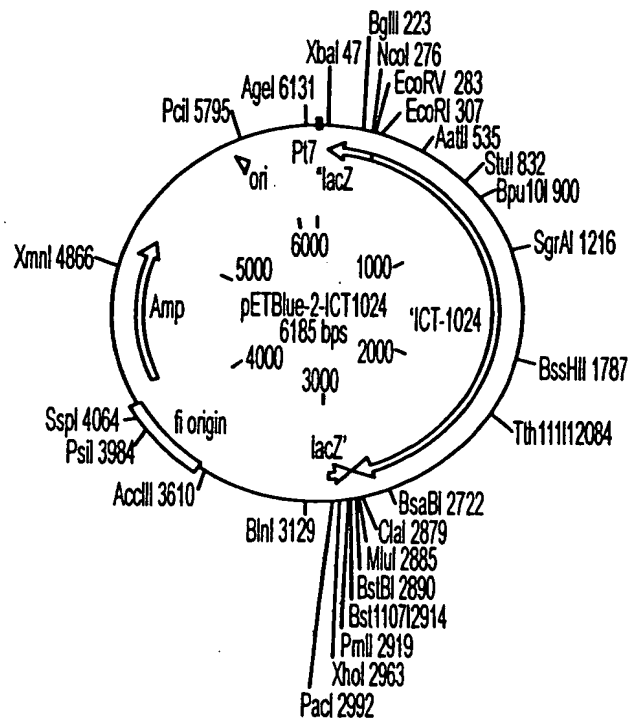
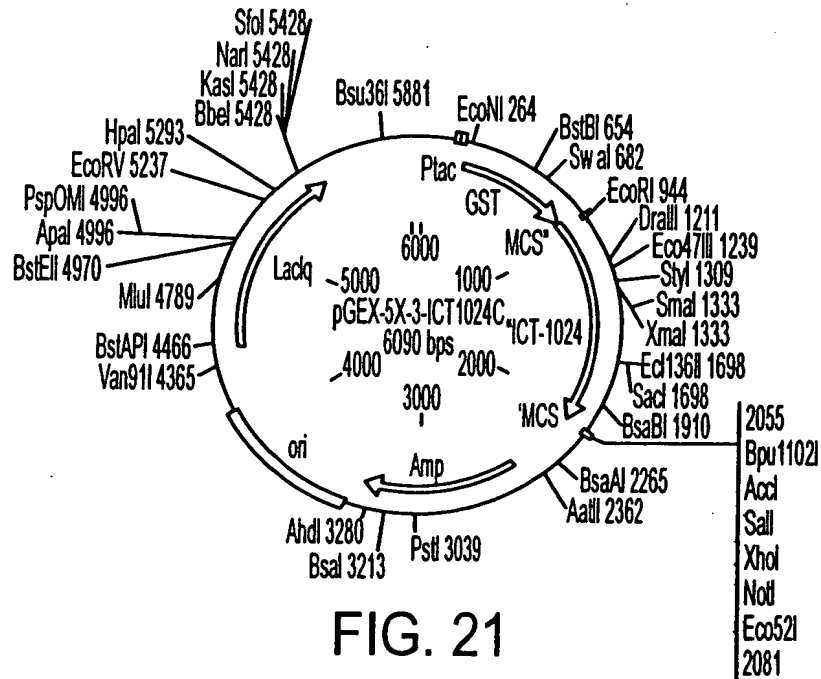


FIG. 20

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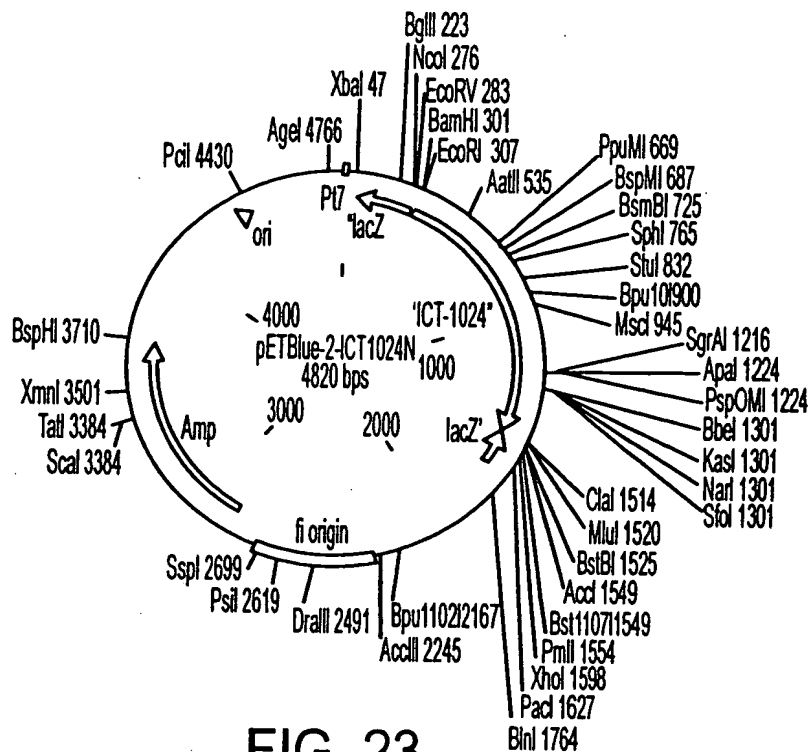


FIG. 23

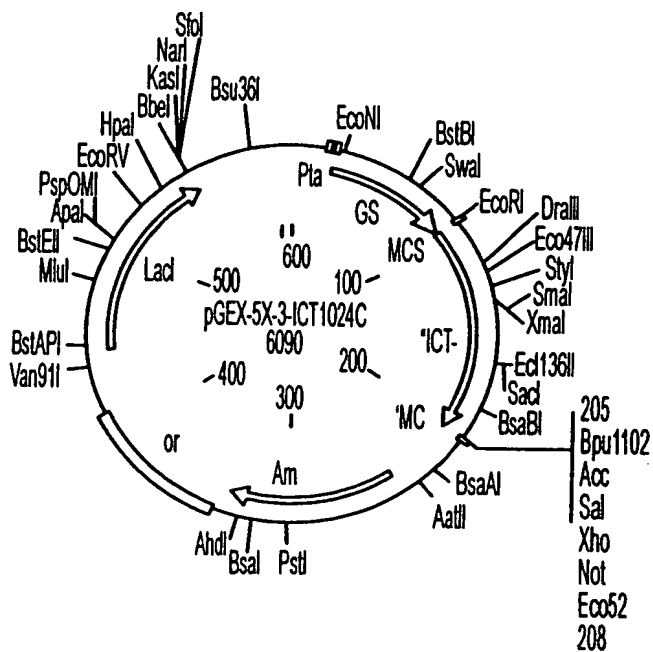


FIG. 24

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(SEQ ID NO:58) ICT1024 PROTEIN (855 AA) CODING REGION: 1670-3637

```
1  TCAATATTGG CCATTAGCCA TATTATTCAT TGGTTATATA GCATAAATCA ATATTGGCTA
61  TTGGCCATTG CATACGTTGT ATCTATATCA TAATATGTAC ATTTATATTG GCTCATGTCC
121 AATATGACCG CCATGTTGGC ATTGATTATT GACTAGTTAT TAATAGTAAT CAATTACGGG
181 GTCATTAGTT CATAGCCCAT ATATGGAGTT CCGCGTTACA TAACTTACGG TAAATGGCCC
241 GCCTGGCTGA CCGCCCAACG ACCCCCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT
301 AGTAACGCCA ATAGGGACTT TCCATTGACG TCAATGGGTG GAGTATTTAC GGTAAACTGC
361 CCACTTGGCA GTACATCAAG TGTATCATAT GCCAAGTCCG CCCCTATTG ACGTCAATGA
421 CGGTAAATGG CCCGCCTGGC ATTATGCCCA GTACATGACC TTACGGGACT TTCCTACTTG
481 GCAGTACATC TACGTATTAG TCATCGCTAT TACCATGGTG ATGCGGTTTT GGCAGTACAC
541 CAATGGGCGT GGATAGCGGT TTGACTCACG GGGATTTCCA AGTCTCCACC CCATTGACGT
601 CAATGGGAGT TTGTTTGGC ACCAAAATCA ACGGGACTTT CCAAATGTC GTAATAACCC
661 CGCCCCGTTG ACGCAAATGG GCGGTAGGCG TGTACGGTGG GAGGTCTATA TAAGCAGAGC
721 TCGTTTAGTG AACCGTCAGA TACTAGAAG CTTTATTGCG GTAGTTTATC ACAGTTAAAT
781 TGCTAACGCA GTCAGTGCTT CTGACACAAC AGTCTCGAAC TTAAGCTGCA GAAGTTGGTC
841 GTGAGGCACT GGGCAGGTAA GTATCAAGGT TACAAGACAG GTTTAAGGAG ACCAATAGAA
901 ACTGGGCTTG TCGAGACAGA GAAGACTCTT GCGTTTCTGA TAGGCACCTA TTGGTCTTAC
961 TGACATCCAC TTTGCCTTTC TCTCCACAGG TGTCCACTCC CAGTTCAATT ACAGCTCTTA
1021 AGGCTAGAGT ACTTAATACG ACTCACTATA GGCTAGCCTC GAGAATTCCA TGAGTGAGGC
1081 CCGCAGGGAC AGCACGAGCA GCCTGCAGCG CAAGAAGCCA CCCTGGCTAA AGCTGGACAT
1141 TCCCTCTGCG GTGCCCCTGA CGGCAGAAGA GCCCAGCTTC CTGCAGCCCC TGAGGCGACA
1201 GGCTTTCCTG AGGAGTGTGA GTATGCCAGC CGAGACAGCC CACATCTCTT CACCCACCA
1261 TGAGCTCCGG CGGCCGGTGC TGCAACGCCA GACGTCCATC ACACAGACCA TCCGCAGGGG
1321 GACCGCCGAC TGGTTTGGAG TGAGCAAGGA CAGTGACAGC ACCCAGAAAT GGCAGCGCAA
1381 GAGCATCCGT CACTGCAGCC AGCGCTACGG GAAGCTGAAG CCCAGGTCC TCCGGGAGCT
1441 GGACCTGCCC AGCCAGGACA ACGTGTGCTG GACCAGCACC GAGACGCCAC CCCACTCTA
1501 CGTGGGGCCA TGCCAGCTGG GCATGCAGAA GATCATAGAC CCCCTGGCCC GTGGCCGTGC
1561 CTTCCGTGTG GCAGATGACA CTGCGGAAGG CCTGAGTGCC CCACACACTC CCGTCACGCC
1621 GGGTGCTGCC TCCCTCTGCT CCTTCTCCAG CTCCCGTCA GGTTCACC GGCTCCCGC
1681 GCGGCGCAAG CGAGAGTCGG TGGCCAAGAT GAGCTTCCGG GCGGCCGAG CGCTGATGAA
1741 AGGCCGCTCC GTTAGGGATG GCACCTTTCG CCGGGCACGG CGTCGAAGCT TCACTCCAGC
1801 TAGCTTCTG GAGGAGGACA CAACTGATTT CCCCATGAG CTGGACACAT CCTTCTTTC
1861 CCGGAAGGT ATCCTCCATG AAGAGCTGTC CACATACCCG GATGAAGTTT TCGAGTCCCC
1921 ATCGGAGGCA GCGCTAAAGG ACTGGGAGAA GGCACCGGAG CAGGCGGACC TCACCGGCGG
1981 GGCCCTGGAC CGCAGCGAGC TTGAGCGCAG CCACCTGATG CTGCCCTTGG AGCGAGGCTG
2041 GCGGAAGCAG AAGGAGGGCG CCGCAGCCCC GCAGCCCAAG GTGCGGCTCC GACAGGAGGT
2101 GGTGAGCACC GCGGGGCCG GACGGGGCCA GCGTATCGCG GTGCCGGTGC GCAAGCTCTT
```

FIG. 25

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2161 CGCCCGGGAG AAGCGGCCGT ATGGGCTGGG CATGGTGGGA CGGCTCACCA ACCGCACCTA
2221 CCGCAAGCGC ATCGACAGCT TCGTCAAGCG CCAGATCGAG GACATGGACG ACCACAGGCC
2281 CTTCTTCACC TACTGGCTTA CCTTCGTGCA CTCGCTCGTC ACCATCCTAG CCGTGTGCAT
2341 CTATGGCATC GCGCCCGTGG GCTTCTCGCA GCATGAGACG GTGGACTCGG TGCTGCGGAA
2401 CCGCGGGGTC TACGAGAACG TCAAGTACGT GCAGCAGGAG AACTTCTGGA TCGGGCCCAG
2461 CTCGGAGGCC CTCATCCACC TGGGCGCCAA GTTTTCGCCC TGCATGCGCC AGGACCCGCA
2521 GGTGCACAGC TTCATTGCTT CGGCGCGCGA GCGCGAGAAG CACTCCGCCT GCTGCGTGCG
2581 CAACGACAGG TCGGGCTGCG TGCAGACCTC GGAGGAGGAG TGCTCGTCCA CGCTGGCAGT
2641 GTGGGTGAAG TGGCCCATCC ATCCCAGCGC CCCAGAGCTT GCGGGCCACA AGAGACAGTT
2701 TGGCTCTGTC TGCCACCAGG ATCCCAGGGT GTGTGATGAG CCCTCCTCCG AAGACCCTCA
2761 TGAGTGGCCA GAAGACATCA CCAAGTGGCC GATCTGCACC AAAAACAGCG CTGGGAACCA
2821 CACCAACCAT CCCACATGG ACTGTGTCAT CACAGGACGG CCCTGCTGCA TTGGCACCAA
2881 GGGCAGGTGT GAGATCACCT CCCGGGAGTA CTGTGACTTC ATGAGGGGCT ACTTCCATGA
2941 GGAGGCCACG CTCTGCTCTC AGGTGCACTG CATGGATGAT GTGTGTGGGC TCCTGCCTTT
3001 TCTCAACCCC GAGGTGCCTG ACCAGTTCTA CCGCCTGTGG CTATCCCTCT TCCTGCACGC
3061 CGGGATCTTG CACTGCCTGG TGCCATCTG CTTCCAGATG ACTGTCCTGC GGGACCTGGA
3121 GAAGCTGGCA GGCTGGCACC GCATAGCCAT CATCTACCTG CTGAGTGGTG TCACCGGCAA
3181 CCTGGCCAGT GCCATCTTCC TGCCATACCG AGCAGAGGTG GGTCCCTGCTG GCTCCCAGTT
3241 CGGCATCCTG GCCTGCCTCT TCGTGGAGCT CTTCCAGAGC TGGCAGATCC TGGCGCGGCC
3301 CTGGCGTGCC TTCTTCAAGC TGCTGGCTGT GGTGCTCTTC CTCTTCACCT TTGGGCTGCT
3361 GCCGTGGATT GACAACTTTG CCCACATCTC GGGGTTTCATC AGTGGCCTCT TCCTCTCCTT
3421 CGCCTTCTTG CCCTACATCA GCTTTGGCAA GTTCGACCTG TACCGGAAAC GCTGCCAGAT
3481 CATCATCTTT CAGGTGGTCT TCCTGGGCCT CCTGGCTGGC CTGGTGGTCC TCTTCTACGT
3541 CTATCCTGTC CGCTGTGAGT GGTGTGAGTT CCTCACCTGC ATCCCCTTCA CTGACAAGTT
3601 CTGTGAGAAG TACGAACTGG ACGCTCAGCT CCACTGAGTC GACCCGGGCG GCCGCTTCGA
3661 GCAGACATGA TAAGATACAT TGATGAGTTT GGACAAACCA CAACTAGAAT GCAGTGAAAA
3721 AAATGCTTTA TTTGTGAAAT TTGTGATGCT ATTGCTTTAT TTGTAACCAT TATAAGCTGC
3781 AATAAACAAAG TTAACAACAA CAATTGCATT CATTTTATGT TTCAGGTTCA GGGGGAGATG
3841 TGGGAGGTTT TTAAAGCAA GTAAAACCTC TACAAATGTG GTAAAATCGA TAAGGATCCG
3901 GGCTGGCGTA ATAGCGAAGA GGCCCGCACC GATCGCCCTT CCCAACAGTT GCGCAGCCTG
3961 AATGGCGAAT GGACGCGCCC TGTAGCGGCG CATTAAGCGC GGCGGGTGTG GTGGTTACGC
4021 GCAGCGTGAC CGCTACACTT GCCAGCGCCC TAGCGCCCGC TCCTTTCGCT TTCTTCCCTT
4081 CCTTTCTCGC CACGTTGCGC GGCTTTCCCC GTCAAGCTCT AAATCGGGGG CTCCCTTTAG
4141 GGTTCCGATT TAGAGCTTTA CGGCACCTCG ACCGCAAAAA ACTTGATTTG GGTGATGGTT
4201 CACGTAGTGG GCCATCGCCC TGATAGACGG TTTTTCGCCC TTTGACGTTG GAGTCCACGT
4261 TCTTTAATAG TGGACTCTTG TTCCAACTG GAACAACACT CAACCCTATC TCGGTCTATT
4321 CTTTTGATTT ATAAGGATT TTGCCGATTT CGGCCTATTG GTTAAAAAAT GAGCTGATTT

FIG. 25 (continued)

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4381 AACAAATATT TAACGCGAAT TTTAACAAAA TATTAACGTT TACAATTTTCG CCTGATGCGG
4441 TATTTTCTCC TTACGCATCT GTGCGGTATT TCACACCGCA TATGGTGCAC TCTCAGTACA
4501 ATCTGCTCTG ATGCCGCATA GTTAAGCCAG CCCCAGACACC CGCCAACACC CGCTGACGCG
4561 CCCTGACGGG CTTGTCTGCT CCCGGCATCC GCTTACAGAC AAGCTGTGAC CGTCTCCGGG
4621 AGCTGCATGT GTCAGAGGTT TTCACCGTCA TCACCGAAAC GCGCGAGACG AAAGGGCCTC
4681 GTGATACGCC TATTTTTATA GGTTAATGTC ATGATAATAA TGGTTTCTTA GACGTCAGGT
4741 GGCACTTTTT GGGGAAATGT GCGCGGAACC CCTATTTGTT TATTTTTCTA AATACATTCA
4801 AATATGTATC CGCTCATGAG ACAATAACCC TGATAAATGC TTCAATAATA TTGAAAAAGG
4861 AAGAGTATGA GTATTCAACA TTTCCGTGTC GCCCTTATTC CCTTTTTTGC GGCATTTTGC
4921 CTTCTGTGTT TTGCTCACCC AGAAACGCTG GTGAAAGTAA AAGATGCTGA AGATCAGTTG
4981 GGTGCACGAG TGGGTTACAT CGAACTGGAT CTCAACAGCG GTAAGATCCT TGAGAGTTTT
5041 CGCCCCGAAG AACGTTTTCC AATGATGAGC ACTTTTAAAG TTCTGCTATG TGGCGCGGTA
5101 TTATCCCGTA TTGACGCCGG GCAAGAGCAA CTCGGTCGCC GCATACACTA TTCTCAGAAT
5161 GACTTG GTTG AGTACTCACC AGTCACAGAA AAGCATCTTA CGGATGGCAT GACAGTAAGA
5221 GAATTATGCA GTGCTGCCAT AACCATGAGT GATAACACTG CGGCCAACTT ACTTCTGACA
5281 ACGATCGGAG GACCGAAGGA GCTAACCGCT TTTTGCACA ACATGGGGGA TCATGTAAGT
5341 CGCCTTGATC GTTGGGAACC GGAGCTGAAT GAAGCCATAC CAAACGACGA GCGTGACACC
5401 ACGATGCCTG TAGCAATGGC AACAACGTTG CGCAAATAT TAACTGGCGA ACTACTTACT
5461 CTAGCTTCCC GGCAACAATT AATAGACTGG ATGGAGGCGG ATAAAGTTGC AGGACCACTT
5521 CTGCGCTCGG CCCTTCCGGC TGGCTGGTTT ATTGCTGATA AATCTGGAGC CGGTGAGCGT
5581 GGGTCTCGCG GTATCATTGC AGCACTGGGG CCAGATGGTA AGCCCTCCCG TATCGTAGTT
5641 ATCTACACGA CGGGGAGTCA GGCAACTATG GATGAACGAA ATAGACAGAT CGCTGAGATA
5701 GGTGCCTCAC TGATTAAGCA TTGGTAACTG TCAGACCAAG TTTACTCATA TATACTTTAG
5761 ATTGATTTAA AACTTCATTT TTAATTTAAA AGGATCTAGG TGAAGATCCT TTTTGATAAT
5821 CTCATGACCA AAATCCCTTA ACGTGAGTTT TCGTTCCACT GAGCGTCAGA CCCCCTAGAA
5881 AAGATCAAAG GATCTTCTTG AGATCCTTTT TTTCTGCGCG TAATCTGCTG CTTGCAACA
5941 AAAAAACCAC CGCTACCAGC GGTGGTTTGT TTGCCGGATC AAGAGCTACC AACTCTTTTT
6001 CCGAAGGTAA CTGGCTTCAG CAGAGCGCAG ATACCAAATA CTGTCCTTCT AGTGTAGCCG
6061 TAGTTAGGCC ACCACTTCAA GAACTCTGTA GCACCGCCTA CATACTCGC TCTGCTAATC
6121 CTGTTACCAG TGGCTGCTGC CAGTGGCGAT AAGTCGTGTC TTACCGGGTT GGACTCAAGA
6181 CGATAGTTAC CGGATAAGGC GCAGCGGTCG GGCTGAACGG GGGGTTCTGT CACACAGCCC
6241 AGCTTGAGC GAACGACCTA CACCGAACTG AGATACCTAC AGCGTGAGCT ATGAGAAAGC
6301 GCCACGCTTC CCGAAGGGAG AAAGGCGGAC AGGTATCCGG TAAGCGGCAG GGTCCGAACA
6361 GGAGAGCGCA CGAGGGAGCT TCCAGGGGGA AACGCCTGGT ATCTTTATAG TCCTGTCTGGG
6421 TTTGCCACC TCTGACTTGA GCGTCGATTT TTGTGATGCT CGTCAGGGGG GCGGAGCCTA
6481 TGGAAAAACG CCAGCAACGC GGCCTTTTTA CGGTTCCCTGG CCTTTTGCTG GCCTTTTGCT
6541 CACATGGCTC GACAGATCT

FIG. 25 (continued)

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(SEQ ID NO:60) ICT1024 N TERMINUS 553 AA CODING REGION: 1070-2731

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1  TCAATATTGG CCATTAGC CA TATTATTCAT TGGTTATATA GCATAAATCA ATATTGGCTA
61  TTGGCCATTG CATACGTTGT ATCTATATCA TAATATGTAC ATTTATATTG GCTCATGTCC
121 AATATGACCG CCATGTTGGC ATTGATTATT GACTAGTTAT TAATAGTAAT CAATTACGGG
181 GTCATTAGTT CATAGCCCAT ATATGGAGTT CCGCGTTACA TAACTTACGG TAAATGGCCC
241 GCCTGGCTGA CCGCCCAACG ACCCCCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT
301 AGTAACGCCA ATAGGGACTT TCCATTGACG TCAATGGGTG GAGTATTTAC GGTAAACTGC
361 CCACTTGGCA GTACATCAAG TGTATCATAT GCCAAGTCCG CCCCTATTG ACGTCAATGA
421 CGGTAAATGG CCCGCCTGGC ATTATGCCCA GTACATGACC TTACGGGACT TTCTACTTG
481 GCAGTACATC TACGTATTAG TCATCGCTAT TACCATGGTG ATGCGGTTTT GGCAGTACAC
541 CAATGGGCGT GGATAGCGGT TTGACTCACG GGGATTTCCA AGTCTCCACC CCATTGACGT
601 CAATGGGAGT TTGTTTTGGC ACCAAAATCA ACGGGACTTT CCAAATGTC GTAATAACCC
661 CGCCCCGTG ACGCAAATGG GCGGTAGGCG TGTACGGTGG GAGGTCTATA TAAGCAGAGC
721 TCGTTTAGTG AACCGTCAGA TCACTAGAAG CTTTATTGCG GTAGTTTATC ACAGTTAAAT
781 TGCTAACGCA GTCAGTGCTT CTGACACAAC AGTCTCGAAC TTAAGCTGCA GAAGTTGGTC
841 GTGAGGCACT GGGCAGGTAA GTATCAAGGT TACAAGACAG GTTTAAGGAG ACCAATAGAA
901 ACTGGGCTTG TCGAGACAGA GAAGACTCTT GCGTTTCTGA TAGGCACCTA TTGGTCTTAC
961 TGACATCCAC TTTGCCTTTC TCTCCACAGG TGTCCACTCC CAGTTCAATT ACAGCTCTTA
1021 AGGCTAGAGT ACTTAATACG ACTCACTATA GGCTAGCCTC GAGAATTCCA TGAGTGAGGC
1081 CCGCAGGGAC AGCACGAGCA GCCTGCAGCG CAAGAAGCCA CCCTGGCTAA AGCTGGACAT
1141 TCCCTCTGCG GTGCCCCTGA CGGCAGAAGA GCCCAGCTTC CTGCAGCCCC TGAGGCGACA
1201 GGCTTTCCTG AGGAGTGTGA GTATGCCAGC CGAGACAGCC CACATCTCTT CACCCACCA
1261 TGAGCTCCGG CGGCCGGTGC TGCAACGCCA GACGTCCATC ACACAGACCA TCCGCAGGGG
1321 GACCGCCGAC TGGTTTGGAG TGAGCAAGGA CAGTGACAGC ACCCAGAAAT GGCAGCGCAA
1381 GAGCATCCGT CACTGCAGCC AGCGTACGG GAAGCTGAAG CCCCAGGTCC TCCGGGAGCT
1441 GGACCTGCCC AGCCAGGACA ACGTGTGCT GACCAGCACC GAGACGCCAC CCCACTCTA
1501 CGTGGGGCCA TGCCAGCTGG GCATGCAGAA GATCATAGAC CCCCTGGCCC GTGGCCGTGC
1561 CTTCCGTGTG GCAGATGACA CTGCGGAAGG CCTGAGTGCC CCACACACTC CCGTCACGCC
1621 GGGTGCTGCC TCCCTCTGCT CCTTCTCCAG CTCCCGCTCA GGTTCACACC GGCTCCCGCG
1681 GCGGCGCAAG CGAGAGTCGG TGGCCAAGAT GAGCTTCCGG GCGGCCGCAG CGCTGATGAA
1741 AGGCCGCTCC GTTAGGGATG GCACCTTTCG CCGGGCACGG CGTCGAAGCT TCACTCCAGC
1801 TAGCTTCTG GAGGAGGACA CAACTGATTT CCCCATGAG CTGGACACAT CCTTCTTTGC
1861 CCGGGAAGGT ATCCTCCATG AAGAGCTGTC CACATACCCG GATGAAGTTT TCGAGTCCCC
1921 ATCGGAGGCA GCGCTAAAGG ACTGGGAGAA GGCACCGGAG CAGGCGGACC TCACCGGCGG
1981 GGCCCTGGAC CGCAGCGAGC TTGAGCGCAG CCACCTGATG CTGCCCTTGG AGCGAGGCTG
2041 GCGGAAGCAG AAGGAGGGCG CCGCAGCCCC GCAGCCCAAG GTGCGGCTCC GACAGGAGGT
2101 GGTGAGCACC GCGGGGCCG GACGGGGCCA GCGTATCGCG GTGCCGGTGC GCAAGCTCTT
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FIG. 26

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2161 CGCCCGGGAG AAGCGGCCGT ATGGGCTGGG CATGGTGGGA CGGCTCACCA ACCGCACCTA
2221 CCGCAAGCGC ATCGACAGCT TCGTCAAGCG CCAGATCGAG GACATGGACG ACCACAGGCC
2281 CTTCTTCACC TACTGGCTTA CCTTCGTGCA CTCGCTCGTC ACCATCCTAG CCGTGTGCAT
2341 CTATGGCATC GCGCCCGTGG GCTTCTCGCA GCATGAGACG GTGGACTCGG TGCTGCGGAA
2401 CCGCGGGGTC TACGAGAACG TCAAGTACGT GCAGCAGGAG AACTTCTGGA TCGGGCCCAG
2461 CTCGGAGGCC CTCATCCACC TGGGCGCCAA GTTTTCGCCC TGCATGCGCC AGGACCCGCA
2521 GGTGCACAGC TTCATTGCTT CGGCGCGCGA GCGCGAGAAG CACTCCGCCT GCTGCGTGCG
2581 CAACGACAGG TCGGGCTGCG TGCAGACCTC GGAGGAGGAG TGCTCGTCCA CGCTGGCAGT
2641 GTGGGTGAAG TGGCCCATCC ATCCCAGCGC CCCAGAGCTT GCGGGCCACA AGAGACAGTT
2701 TGGCTCTGTC TGCCACCAGG ATCCCAGGTG AGTCGACCCG GGCGGCCGCT TCGAGCAGAC
2761 ATGATAAGAT ACATTGATGA GTTTGGACAA ACCACAATA GAATGCAGTG AAAAAAATGC
2821 TTTATTTGTG AAATTTGTGA TGCTATTGCT TTATTTGTAA CCATTATAAG CTGCAATAAA
2881 CAAGTTAACA ACAACAATTG CATTCATTTT ATGTTTCAGG TTCAGGGGGA GATGTGGGAG
2941 GTTTTTTTAAA GCAAGTAAAA CCTCTACAAA TGTGGTAAAA TCGATAAGGA TCCGGGCTGG
3001 CGTAATAGCG AAGAGGCCCG CACCGATCGC CCTTCCCAAC AGTTGCGCAG CCTGAATGGC
3061 GAATGGACGC GCCCTGTAGC GGCGCATTAA GCGCGGCGGG TGTGGTGGTT ACGCGCAGCG
3121 TGACCGCTAC ACTTGCCAGC GCCCTAGCGC CCGCTCCTTT CGCTTTCTTC CCTTCCTTTC
3181 TCGCCACGTT CGCCGGCTTT CCCCGTCAAG CTCTAAATCG GGGGCTCCCT TTAGGGTTCC
3241 GATTTAGAGC TTTACGGCAC CTCGACCGCA AAAAAGTTGA TTTGGGTGAT GGTTACGTA
3301 GTGGGCCATC GCCCTGATAG ACGGTTTTTC GCCCTTTGAC GTTGGAGTCC ACGTTCCTTA
3361 ATAGTGGACT CTTGTTCCAA ACTGGAACAA CACTCAACCC TATCTCGGTC TATTCTTTTG
3421 ATTTATAAGG GATTTTGCCG ATTCGGCCT ATTGGTTAAA AAATGAGCTG ATTTAACAAA
3481 TATTTAACGC GAATTTTAAC AAAATATTAA CGTTTACAAT TTCGCCTGAT GCGGTATTTT
3541 CTCCTTACGC ATCTGTGCGG TATTTACAC CGCATATGGT GCACTCTCAG TACAATCTGC
3601 TCTGATGCCG CATAGTTAAG CCAGCCCCGA CACCCGCCAA CACCCGCTGA CGCGCCCTGA
3661 CGGGCTTGTC TGCTCCCGGC ATCCGCTTAC AGACAAGCTG TGACCGTCTC CGGGAGCTGC
3721 ATGTGTCAGA GGTTTTCACC GTCATCACCG AAACGCGCGA GACGAAAGGG CCTCGTGATA
3781 CGCCTATTTT TATAGGTTAA TGTCATGATA ATAATGGTTT CTTAGACGTC AGGTGGCACT
3841 TTTCGGGGAA ATGTGCGCGG AACCCCTATT TGTTTATTTT TCTAAATACA TTCAAATATG
3901 TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA AAGGAAGAGT
3961 ATGAGTATTC AACATTTCCG TGTCGCCCTT ATTCCCTTTT TTGCGGCATT TTGCCTTCCT
4021 GTTTTTGCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG CTGAAGATCA GTTGGGTGCA
4081 CGAGTGGGTT ACATCGAACT GGATCTCAAC AGCGGTAAGA TCCTTGAGAG TTTTCGCCCC
4141 GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCTGC TATGTGGCGC GGTATTATCC
4201 CGTATTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG
4261 GTTGAGTACT CACCAGTCAC AGAAAAGCAT CTTACGGATG GCATGACAGT AAGAGAATTA
4321 TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA ACTTACTTCT GACAACGATC

FIG. 26 (continued)

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4381 GGAGGACCGA AGGAGCTAAC CGCTTTTTTG CACAACATGG GGGATCATGT AACTCGCCTT
4441 GATCGTTGGG AACCGGAGCT GAATGAAGCC ATACCAAACG ACGAGCGTGA CACCACGATG
4501 CCTGTAGCAA TGGCAACAAC GTTGCGCAA CTATTAAGTG GCGAACTACT TACTCTAGCT
4561 TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG TTGCAGGACC ACTTCTGCGC
4621 TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT
4681 CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT AGTTATCTAC
4741 ACGACGGGGA GTCAGGCAAC TATGGATGAA CGAAATAGAC AGATCGCTGA GATAGGTGCC
4801 TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT CATATATACT TTAGATTGAT
4861 TTAAAACTTC ATTTTAAATT TAAAAGGATC TAGGTGAAGA TCCTTTTTTGA TAATCTCATG
4921 ACCAAAATCC CTTAACGTGA GTTTTCGTTT CACTGAGCGT CAGACCCCGT AGAAAAGATC
4981 AAAGGATCTT CTTGAGATCC TTTTTTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA
5041 CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT TTTTCCGAAG
5101 GTAAGTGGCT TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA
5161 GGCCACCACT TCAAGAACTC TGTAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTTA
5221 CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTTGGACTC AAGACGATAG
5281 TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGCACACA GCCCAGCTTG
5341 GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA AAGCGCCACG
5401 CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG
5461 CGCACGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT ATAGTCCTGT CGGGTTTCGC
5521 CACCTCTGAC TTGAGCGTCG ATTTTGTGA TGCTCGTCAG GGGGGCGGAG CCTATGGAAA
5581 AACGCCAGCA ACGCGGCCTT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG
5641 GCTCGACAGA TCT
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FIG. 26 (continued)

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(SEQ ID NO: 61) ICT1024 coding region: 947-3518

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1   TCGACTCGAG CGGCCGCATC GTGACTGACT GACGATCTGC CTCGCGCGTT TCGGTGATGA
61  CGGTGAAAAC CTCTGACACA TGCAGCTCCC GGAGACGGTC ACAGCTTGTC TGTAAGCGGA
121 TGCCGGGAGC AGACAAGCCC GTCAGGGCGC GTCAGCGGGT GTTGGCGGGT GTCGGGGCGC
181 AGCCATGACC CAGTCACGTA GCGATAGCGG AGTGTATAAT TCTTGAAGAC GAAAGGGCCT
241 CGTGATACGC CTATTTTAT AGGTAAATGT CATGATAATA ATGGTTTCTT AGACGTCAGG
301 TGGCACTTTT CGGGGAAATG TGCGCGGAAC CCCTATTTGT TTATTTTCTT AAATACATTC
361 AAATATGTAT CCGCTCATGA GACAATAACC CTGATAAATG CTTCAATAAT ATTGAAAAAG
421 GAAGAGTATG AGTATTCAAC ATTTCCGTGT CGCCCTTATT CCCTTTTTTG CGGCATTTTG
481 CCTTCCTGTT TTTGCTCACC CAGAAACGCT GGTGAAAGTA AAAGATGCTG AAGATCAGTT
541 GGGTGCACGA GTGGGTTACA TCGAACTGGA TCTCAACAGC GGTAAGATCC TTGAGAGTTT
601 TCGCCCCGAA GAACGTTTTT CAATGATGAG CACTTTTAAA GTTCTGCTAT GTGGCGCGGT
661 ATTATCCCGT GTTGACGCCG GGCAAGAGCA ACTCGGTCGC CGCATACACT ATTCTCAGAA
721 TGACTTGGTT GAGTACTCAC CAGTCACAGA AAAGCATCTT ACGGATGGCA TGACAGTAAG
781 AGAATTATGC AGTGCTGCCA TAACCATGAG TGATAACACT GCGGCCAACT TACTTCTGAC
841 AACGATCGGA GGACCGAAGG AGCTAACCGC TTTTTTGCAC AACATGGGGG ATCATGTAAC
901 TCGCCTTGAT CGTTGGGAAC CGGAGCTGAA TGAAGCCATA CCAAACGACG AGCGTGACAC
961 CACGATGCCT GCAGCAATGG CAACAACGTT GCGCAAATA TTAAGTGGCG AACTACTTAC
1021 TCTAGCTTCC CGGCAACAAT TAATAGACTG GATGGAGGCG GATAAAGTTG CAGGACCACT
1081 TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT TATTGCTGAT AAATCTGGAG CCGGTGAGCG
1141 TGGGTCTCGC GGTATCATTG CAGCACTGGG GCCAGATGGT AAGCCCTCCC GTATCGTAGT
1201 TATCTACACG ACGGGGAGTC AGGCAACTAT GGATGAACGA AATAGACAGA TCGCTGAGAT
1261 AGGTGCCTCA CTGATTAAGC ATTGGTAACT GTCAGACCAA GTTTACTCAT ATATACTTTA
1321 GATTGATTTA AAACCTCATT TTAAATTTAA AAGGATCTAG GTGAAGATCC TTTTGTATAA
1381 TCTCATGACC AAAATCCCTT AACGTGAGTT TTCGTTCCAC TGAGCGTCAG ACCCCGTAGA
1441 AAAGATCAAA GGATCTTCTT GAGATCCTTT TTTTCTGCGC GTAATCTGCT GCTTGCAAAC
1501 AAAAAAACCA CCGCTACCAG CGGTGGTTTG TTTGCCGGAT CAAGAGCTAC CAACTCTTTT
1561 TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAAT ACTGTCCTTC TAGTGTAGCC
1621 GTAGTTAGGC CACCACTTCA AGAACTCTGT AGCACCGCCT ACATACCTCG CTCTGCTAAT
1681 CCTGTTACCA GTGGCTGCTG CCAGTGGCGA TAAGTCGTGT CTTACCGGGT TGGACTCAAG
1741 ACGATAGTTA CCGGATAAGG CGCAGCGGTC GGGCTGAACG GGGGGTTTCG GCACACAGCC
1801 CAGCTTGAGG CGAACGACCT ACACCGAACT GAGATACCTA CAGCGTGAGC TATGAGAAAG
1861 CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA GGGTCGGAAC
1921 AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG TATCTTTATA GTCCTGTCGG
1981 GTTTCGCCAC CTCTGACTTG AGCGTCGATT TTTGTGATGC TCGTCAGGGG GGCGGAGCCT
2041 ATGGAAAAAC GCCAGCAACG CGGCCTTTTT ACGGTTCTTG GCCTTTTGCT GGCCTTTTGC
2101 TCACATGTTC TTTCTGCGT TATCCCCTGA TTCTGTGGAT AACCGTATTA CCGCCTTTGA
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FIG. 27

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2161 GTGAGCTGAT ACCGCTCGCC GCAGCCGAAC GACCGAGCGC AGCGAGTCAG TGAGCGAGGA
2221 AGCGGAAGAG CGCCTGATGC GGTATTTTCT CCTTACGCAT CTGTGCGGTA TTTCACACCG
2281 CATAAATTCC GACACCATCG AATGGTGCAA AACCTTTCGC GGTATGGCAT GATAGCGCCC
2341 GGAAGAGAGT CAATTCAGGG TGGTGAATGT GAAACCAGTA ACGTTATACG ATGTCGCAGA
2401 GTATGCCGGT GTCTCTTATC AGACCGTTTC CCGCGTGGTG AACCAGGCCA GCCACGTTTC
2461 TGCGAAAACG CGGGAAAAG TGGAAGCGGC GATGGCGGAG CTGAATTACA TTCCCAACCG
2521 CGTGGCACA CAACTGGCGG GCAAACAGTC GTTGCTGATT GGCGTTGCCA CCTCCAGTCT
2581 GGCCCTGCAC GCGCCGTCGC AAATTGTCGC GGCGATTAAA TCTCGCGCCG ATCAACTGGG
2641 TGCCAGCGTG GTGGTGTCTA TGGTAGAACG AAGCGGCGTC GAAGCCTGTA AAGCGGCGGT
2701 GCACAATCTT CTCGCGCAAC GCGTCAGTGG GCTGATCATT AACTATCCGC TGGATGACCA
2761 GGATGCCATT GCTGTGGAAG CTGCCTGCAC TAATGTTCCG GCGTTATTTT TTGATGTCTC
2821 TGACCAGACA CCCATCAACA GTATTATTTT CTCCCATGAA GACGGTACGC GACTGGGCGT
2881 GGAGCATCTG GTCGCATTGG GTCACCAGCA AATCGCGCTG TTAGCGGGCC CATTAAAGTTC
2941 TGTCTCGGCG CGTCTGCGTC TGGCTGGCTG GCATAAATAT CTCACTCGCA ATCAAATTCA
3001 GCCGATAGCG GAACGGGAAG GCGACTGGAG TGCCATGTCC GGTTTTCAAC AAACCATGCA
3061 AATGCTGAAT GAGGGCATCG TTCCCACTGC GATGCTGGTT GCCAACGATC AGATGGCGCT
3121 GGGCGCAATG CGCGCCATTA CCGAGTCCGG GCTGCGCGTT GGTGCGGATA TCTCGGTAGT
3181 GGGATACGAC GATACCGAAG ACAGCTCATG TTATATCCCG CCGTTAACCA CCATCAAACA
3241 GGATTTTCGC CTGCTGGGGC AAACCAGCGT GGACCGCTTG CTGCAACTCT CTCAGGGCCA
3301 GGCGGTGAAG GGCAATCAGC TGTTGCCCCG CTCACTGGTG AAAAGAAAAA CCACCCTGGC
3361 GCCCAATACG CAAACCGCCT CTCCCCGCGC GTTGGCCGAT TCATTAATGC AGCTGGCACG
3421 ACAGGTTTCC CGACTGGAAA GCGGGCAGTG AGCGCAACGC AATTAATGTG AGTTAGCTCA
3481 CTCATTAGGC ACCCCAGGCT TTACACTTTA TGCTTCCGGC TCGTATGTTG TGTGGAATTG
3541 TGAGCGGATA ACAATTTTAC ACAGGAAACA GCTATGACCA TGATTACGGA TTCACTGGCC
3601 GTCGTTTTAC AACGTCGTGA CTGGGAAAAC CCTGGCGTTA CCCAACTTAA TCGCCTTGCA
3661 GCACATCCCC CTTTCGCCAG CTGGCGTAAT AGCGAAGAGG CCCGCACCGA TCGCCCTTCC
3721 CAACAGTTGC GCAGCCTGAA TGGCGAATGG CGCTTTGCCT GGTTCCTGGC ACCAGAAGCG
3781 GTGCCGGAAA GCTGGCTGGA GTGCGATCTT CCTGAGGCCG ATACTGTCTG CGTCCCCTCA
3841 AACTGGCAGA TGCACGGTTA CGATGCGCCC ATCTACACCA ACGTAACCTA TCCCATTACG
3901 GTCAATCCGC CGTTTGTTC CACGGAGAAT CCGACGGGT GTTACTCGCT CACATTTAAT
3961 GTTGATGAAA GCTGGCTACA GGAAGGCCAG ACGCGAATTA TTTTGTATGG CGTTGGAATT
4021 AGCTTATCGA CTGCACGGTG CACCAATGCT TCTGGCGTCA GGCAGCCATC GGAAGCTGTG
4081 GTATGGCTGT GCAGGTCGTA AATCACTGCA TAATTCGTGT CGCTCAAGGC GCACTCCCGT
4141 TCTGGATAAT GTTTTTTGCG CCGACATCAT AACGGTTCTG GCAAATATTC TGAAATGAGC
4201 TGTTGACAAT TAATCATCGG CTCGTATAAT GTGTGGAATT GTGAGCGGAT AACAATTTCA
4261 CACAGGAAAC AGTATTCATG TCCCCTATAC TAGGTTATTG GAAAATTAAG GGCCTTGTGC
4321 AACCCACTCG ACTTCTTTTG GAATATCTTG AAGAAAAATA TGAAGAGCAT TTGTATGAGC

FIG. 27 (continued)

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4381 GCGATGAAGG TGATAAATGG CGAAACAAAA AGTTTGAATT GGGTTTGGAG TTTCCCAATC
4441 TTCCTTATTA TATTGATGGT GATGTTAAAT TAACACAGTC TATGGCCATC ATACGTTATA
4501 TAGCTGACAA GCACAACATG TTGGGTGGTT GTCCAAAAGA GCGTGCAGAG ATTTCAATGC
4561 TTGAAGGAGC GGTTTTGGAT ATTAGATACG GTGTTTCGAG AATTGCATAT AGTAAAGACT
4621 TTGAAACTCT CAAAGTTGAT TTTCTTAGCA AGCTACCTGA AATGCTGAAA ATGTTCGAAG
4681 ATCGTTTATG TCATAAAACA TATTTAAATG GTGATCATGT AACCCATCCT GACTTCATGT
4741 TGTATGACGC TCTTGATGTT GTTTTATACA TGGACCCAAT GTGCCTGGAT GCGTTCCCAA
4801 AATTAGTTTG TTTTAAAAAA CGTATTGAAG CTATCCCACA AATTGATAAG TACTTGAAAT
4861 CCAGCAAGTA TATAGCATGG CCTTTGCAGG GCTGGCAAGC CACGTTTGGT GGTGGCGACC
4921 ATCCTCCAAA ATCGGATCTG ATCGAAGGTC GTGGGATCCC CAGG

FIG. 27 (continued)

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(SEQ ID NO: 62) ICT1024 N terminus 553 aa coding region: 947-2600

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1  AGCTTATCGA CTGCACGGTG CACCAATGCT TCTGGCGTCA GGCAGCCATC GGAAGCTGTG
61  GTATGGCTGT GCAGGTCGTA AATCACTGCA TAATTCGTGT CGCTCAAGGC GCACTCCCGT
121 TCTGGATAAT GTTTTTTGCG CCGACATCAT AACGGTTCTG GCAAATATTC TGAAATGAGC
181 TGTTGACAAT TAATCATCGG CTCGTATAAT GTGTGGAATT GTGAGCGGAT AACAATTTCA
241 CACAGGAAAC AGTATTCATG TCCCCTATAC TAGGTTATTG GAAAATTAAG GGCCTTGTGC
301 AACCCACTCG ACTTCTTTTG GAATATCTTG AAGAAAAATA TGAAGAGCAT TTGTATGAGC
361 GCGATGAAGG TGATAAATGG CGAAACAAAA AGTTTGAATT GGGTTTGGAG TTTCCCAATC
421 TTCCTTATTA TATTGATGGT GATGTTAAAT TAACACAGTC TATGGCCATC ATACGTTATA
481 TAGCTGACAA GCACAACATG TTGGGTGGTT GTCCAAAAGA GCGTGCAGAG ATTTCAATGC
541 TTGAAGGAGC GGTTTTGGAT ATTAGATACG GTGTTTCGAG AATTGCATAT AGTAAAGACT
601 TTGAAACTCT CAAAGTTGAT TTTCTTAGCA AGCTACCTGA AATGCTGAAA ATGTTCGAAG
661 ATCGTTTATG TCATAAAACA TATTTAAATG GTGATCATGT AACCCATCCT GACTTCATGT
721 TGTATGACGC TCTTGATGTT GTTTTATACA TGGACCCAAT GTGCCTGGAT GCGTTCCCAA
781 AATTAGTTTG TTTTAAAAAA CGTATTGAAG CTATCCCACA AATTGATAAG TACTTGAAAT
841 CCAGCAAGTA TATAGCATGG CCTTTGCAGG GCTGGCAAGC CACGTTTGGT GGTGGCGACC
901 ATCCTCCAAA ATCGGATCTG ATCGAAGGTC GTGGGATCCC CAGGAATTCC ATGAGTGAGG
961 CCCGCAGGGA CAGCACGAGC AGCCTGCAGC GCAAGAAGCC ACCCTGGCTA AAGCTGGACA
1021 TTCCCTCTGC GGTGCCCCCTG ACGGCAGAAG AGCCCAGCTT CCTGCAGCCC CTGAGGCGAC
1081 AGGCTTTCCT GAGGAGTGTG AGTATGCCAG CCGAGACAGC CCACATCTCT TCACCCACC
1141 ATGAGCTCCG GCGGCCGGTG CTGCAACGCC AGACGTCCAT CACACAGACC ATCCGCAGGG
1201 GGACCGCCGA CTGGTTTGA GTGAGCAAGG ACAGTGACAG CACCCAGAAA TGGCAGCGCA
1261 AGAGCATCCG TCACTGCAGC CAGCGCTACG GGAAGCTGAA GCCCCAGGTC CTCCGGGAGC
1321 TGGACCTGCC CAGCCAGGAC AACGTGTCGC TGACCAGCAC CGAGACGCCA CCCCCTCTCT
1381 ACGTGGGGCC ATGCCAGCTG GGCATGCAGA AGATCATAGA CCCCCTGGCC CGTGGCCGTG
1441 CCTTCCGTGT GGCAGATGAC ACTGCGGAAG GCCTGAGTGC CCCACACACT CCCGTCACGC
1501 CGGGTGCTGC CTCCCTCTGC TCCTTCTCCA GCTCCCCTC AGGTTTCCAC CGGCTCCCGC
1561 GCGGGCGCAA GCGAGAGTCG GTGGCCAAGA TGAGCTTCCG GGCGGCCGCA GCGCTGATGA
1621 AAGGCCGCTC CGTTAGGGAT GGCACCTTTC GCCGGGCACG GCGTCGAAGC TTCCTCCAG
1681 CTAGCTTTCT GGAGGAGGAC ACAACTGATT TCCCCGATGA GCTGGACACA TCCTTCTTTG
1741 CCCGGAAGG TATCCTCCAT GAAGAGCTGT CCACATACCC GGATGAAGTT TTCGAGTCCC
1801 CATCGGAGGC AGCGCTAAAG GACTGGGAGA AGGCACCGGA GCAGGCGGAC CTCACCGGCG
1861 GGGCCCTGGA CCGCAGCGAG CTTGAGCGCA GCCACCTGAT GCTGCCCTTG GAGCGAGGCT
1921 GCGGAAGCA GAAGGAGGGC GCCGCAGCCC CGCAGCCCAA GGTGCGGCTC CGACAGGAGG
1981 TGGTGAGCAC CGCGGGGCCG CGACGGGGCC AGCGTATCGC GGTGCCGGTG CGCAAGCTCT
2041 TCGCCCGGGA GAAGCGGCCG TATGGGCTGG GCATGGTGGG ACGGCTCACC AACCGCACCT
2101 ACCGCAAGCG CATCGACAGC TTCGTCAAGC GCCAGATCGA GGACATGGAC GACCACAGGC
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FIG. 28

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2161 CCTTCTTCAC CTA CTACTGGCTT ACCTTCGTGC ACTCGCTCGT CACCATCCTA GCCGTGTGCA
2221 TCTATGGCAT CGCGCCCGTG GGCTTCTCGC AGCATGAGAC GGTGGACTCG GTGCTGCGGA
2281 ACCGCGGGGT CTACGAGAAC GTCAAGTACG TGCAGCAGGA GAACTTCTGG ATCGGGCCCCA
2341 GCTCGGAGGC CCTCATCCAC CTGGGCGCCA AGTTTTTCGCC CTGCATGCGC CAGGACCCGC
2401 AGGTGCACAG CTTTCATTCGC TCGGCGCGCG AGCGCGAGAA GCACTCCGCC TGCTGCGTGC
2461 GCAACGACAG GTCGGGCTGC GTGCAGACCT CGGAGGAGGA GTGCTCGTCC ACGCTGGCAG
2521 TGTGGGTGAA GTGGCCCATC CATCCCAGCG CCCAGAGCT TCGGGGCCAC AAGAGACAGT
2581 TTGGCTCTGT CTGCCACCAG GATCCCAGGT GAGTCGACTC GAGCGGCCGC ATCGTGACTG
2641 ACTGACGATC TGCCTCGCGC GTTTCGGTGA TGACGGTGAA AACCTCTGAC ACATGCAGCT
2701 CCCGGAGACG GTCACAGCTT GTCTGTAAGC GGATGCCGGG AGCAGACAAG CCCGTCAGGG
2761 CGCGTCAGCG GGTGTTGGCG GGTGTCGGGG CGCAGCCATG ACCCAGTCAC GTAGCGATAG
2821 CGGAGTGTAT AATTCTTGAA GACGAAAGGG CCTCGTGATA CGCCTATTTT TATAGGTTAA
2881 TGTCATGATA ATAATGGTTT CTTAGACGTC AGGTGGCACT TTTCGGGGAA ATGTGCGCGG
2941 AACCCTATT TGTATTTTT TCTAAATACA TTCAAATATG TATCCGCTCA TGAGACAATA
3001 ACCCTGATAA ATGCTTCAAT AATATTGAAA AAGGAAGAGT ATGAGTATTC AACATTTCCG
3061 TGTCGCCCTT ATTCCTTTTT TTGCGGCATT TTGCCTTCCT GTTTTTGCTC ACCCAGAAAC
3121 GCTGGTGAAA GTAAAAGATG CTGAAGATCA GTTGGGTGCA CGAGTGGGTT ACATCGAACT
3181 GGATCTCAAC AGCGGTAAGA TCCTTGAGAG TTTTCGCCCC GAAGAACGTT TTCCAATGAT
3241 GAGCACTTTT AAAGTTCTGC TATGTGGCGC GGTATTATCC CGTGTTGACG CCGGGCAAGA
3301 GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG GTTGAGTACT CACCAGTCAC
3361 AGAAAAGCAT CTTACGGATG GCATGACAGT AAGAGAATTA TGCAGTGCTG CCATAACCAT
3421 GAGTGATAAC ACTGCGGCCA ACTTACTTCT GACAACGATC GGAGGACCGA AGGAGCTAAC
3481 CGCTTTTTTG CACAACATGG GGGATCATGT AACTCGCCTT GATCGTTGGG AACC GGAGCT
3541 GAATGAAGCC ATACCAAACG ACGAGCGTGA CACCACGATG CCTGCAGCAA TGGCAACAAC
3601 GTTGCGCAA CTATTAAGT GCGAACTACT TACTCTAGCT TCCCGGCAAC AATTAATAGA
3661 CTGGATGGAG GCGGATAAAG TTGCAGGACC ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG
3721 GTTTATTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT CGCGGTATCA TTGCAGCACT
3781 GGGGCCAGAT GGTAAGCCCT CCCGTATCGT AGTTATCTAC ACGACGGGGA GTCAGGCAAC
3841 TATGGATGAA CGAAATAGAC AGATCGCTGA GATAGGTGCC TCACTGATTA AGCATTGGTA
3901 ACTGTCAGAC CAAGTTTACT CATATATACT TTAGATTGAT TTAAAACTTC ATTTTAAATT
3961 TAAAAGGATC TAGGTGAAGA TCCTTTTTGA TAATCTCATG ACCAAAATCC CTTAACGTGA
4021 GTTTTCGTTT CACTGAGCGT CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGATCC
4081 TTTTTTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT
4141 TTGTTTGCCG GATCAAGAGC TACCAACTCT TTTTCCGAAG GTAAGTGGCT TCAGCAGAGC
4201 GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA GGCCACCACT TCAAGAACTC
4261 TGTAGACCG CCTACATACC TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG
4321 CGATAAGTCG TGTCTTACCG GGTTGGACTC AAGACGATAG TTACCGGATA AGGCGCAGCG

FIG. 28 (continued)

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4381 GTCGGGCTGA ACGGGGGGTT CGTGACACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA
4441 ACTGAGATAC CTACAGCGTG AGCTATGAGA AAGCGCCACG CTTCCCGAAG GGAGAAAGGC
4501 GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG CGCACGAGGG AGCTTCCAGG
4561 GGGAAACGCC TGGTATCTTT ATAGTCCTGT CGGGTTTCGC CACCTCTGAC TTGAGCGTCG
4621 ATTTTTGTGA TGCTCGTCAG GGGGGCGGAG CCTATGGAAA AACGCCAGCA ACGCGGCCTT
4681 TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG TTCTTTCCTG CGTTATCCCC
4741 TGATTCTGTG GATAACCGTA TTACCGCCTT TGAGTGAGCT GATACCGCTC GCCGCAGCCG
4801 AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA GAGCGCCTGA TGCGGTATTT
4861 TCTCCTTACG CATCTGTGCG GTATTTTACA CCGCATAAAT TCCGACACCA TCGAATGGTG
4921 CAAAACCTTT CGCGGTATGG CATGATAGCG CCCGGAAGAG AGTCAATTCA GGGTGGTGAA
4981 TGTGAAACCA GTAACGTTAT ACGATGTCGC AGAGTATGCC GGTGTCTCTT ATCAGACCGT
5041 TTCCCGCGTG GTGAACCAGG CCAGCCACGT TTCTGCGAAA ACGCGGGAAA AAGTGGAAGC
5101 GGCGATGGCG GAGCTGAATT ACATTCCCAA CCGCGTGGCA CAACAACTGG CGGGCAAACA
5161 GTCGTTGCTG ATTGGCGTTG CCACCTCCAG TCTGGCCCTG CACGCGCCGT CGCAAATTGT
5221 CGCGGCGATT AAATCTCGCG CCGATCAACT GGGTGCCAGC GTGGTGGTGT CGATGGTAGA
5281 ACGAAGCGGC GTCGAAGCCT GTAAAGCGGC GGTGCACAAT CTTCTCGCGC AACCGGTCAG
5341 TGGGCTGATC ATTAACATATC CGCTGGATGA CCAGGATGCC ATTGCTGTGG AAGCTGCCTG
5401 CACTAATGTT CCGGCGTTAT TTCTTGATGT CTCTGACCAG ACACCCATCA ACAGTATTAT
5461 TTTCTCCCAT GAAGACGGTA CGCGACTGGG CGTGGAGCAT CTGGTCGCAT TGGGTCACCA
5521 GCAAATCGCG CTGTTAGCGG GCCCATTAAG TTCTGTCTCG GCGCGTCTGC GTCTGGCTGG
5581 CTGGCATAAA TATCTCACTC GCAATCAAAT TCAGCCGATA GCGGAACGGG AAGGCGACTG
5641 GAGTGCCATG TCCGGTTTTT AACAAACCAT GCAAATGCTG AATGAGGGCA TCGTTCCCAC
5701 TGCGATGCTG GTTGCCAACG ATCAGATGGC GCTGGGCGCA ATGCGCGCCA TTACCGAGTC
5761 CGGGCTGCGC GTTGGTGCGG ATATCTCGGT AGTGGGATAC GACGATACCG AAGACAGCTC
5821 ATGTTATATC CCGCCGTTAA CCACCATCAA ACAGGATTTT CGCCTGCTGG GGCAAACCAG
5881 CGTGGACCGC TTGCTGCAAC TCTCTAGGG CCAGGCGGTG AAGGGCAATC AGCTGTTGCC
5941 CGTCTCACTG GTGAAAAGAA AAACCACCCT GGCGCCAAT ACGCAAACCG CCTCTCCCCG
6001 CGCGTTGGCC GATTCATTAA TGCAGCTGGC ACGACAGGTT TCCCGACTGG AAAGCGGGCA
6061 GTGAGCGCAA CGCAATTAAT GTGAGTTAGC TCACTCATTA GGCACCCAG GCTTTACT
6121 TTATGCTTCC GGCTCGTATG TTGTGTGGAA TTGTGAGCGG ATAACAATTT CACACAGGAA
6181 ACAGCTATGA CCATGATTAC GGATTCCTG GCCGTCGTTT TACAACGTCG TGA CTGGGAA
6241 AACCTGGCG TTACCAACT TAATCGCCTT GCAGCACATC CCCCTTTCGC CAGCTGGCGT

FIG. 28 (continued)

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6301 AATAGCGAAG AGGCCCCGCAC CGATCGCCCT TCCCAACAGT TGC GCAGCCT GAATGGCGAA
6361 TGGCGCTTTG CCTGGTTTCC GGCACCAGAA GCGGTGCCGG AAAGCTGGCT GGAGTGCGAT
6421 CTTCTGAGG CCGATACTGT CGTCGTCCCC TCAAAGTGGC AGATGCACGG TTACGATGCG
6481 CCCATCTACA CCAACGTAAC CTATCCCATT ACGGTCAATC CGCCGTTTGT TCCCACGGAG
6541 AATCCGACGG GTTGTTACTC GCTCACATTT AATGTTGATG AAAGCTGGCT ACAGGAAGGC
6601 CAGACGCGAA TTATTTTTGA TGGCGTTGGA ATT

FIG. 28 (continued)

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(SEQ ID NO:64) Coding region for the C terminus 375 aa: 945-2069

```
1  AGCTTATCGA CTGCACGGTG CACCAATGCT TCTGGCGTCA GGCAGCCATC GGAAGCTGTG
61  GTATGGCTGT GCAGGTCGTA AATCACTGCA TAATTCGTGT CGCTCAAGGC GCACTCCCGT
121 TCTGGATAAT GTTTTTTGCG CCGACATCAT AACGGTTCTG GCAAATATTC TGAAATGAGC
181 TGTTGACAAT TAATCATCGG CTCGTATAAT GTGTGGAATT GTGAGCGGAT AACAAATTCA
241 CACAGGAAAC AGTATTCATG TCCCCTATAC TAGGTTATTG GAAAATTAAG GGCCTTGTGC
301 AACCCACTCG ACTTCTTTTG GAATATCTTG AAGAAAAATA TGAAGAGCAT TTGTATGAGC
361 GCGATGAAGG TGATAAATGG CGAAACAAAA AGTTTGAATT GGGTTTGGAG TTTCCCAATC
421 TTCCTTATTA TATTGATGGT GATGTTAAAT TAACACAGTC TATGGCCATC ATACGTTATA
481 TAGCTGACAA GCACAACATG TTGGGTGGTT GTCCAAAAGA GCGTGCGAGG ATTTCAATGC
541 TTGAAGGAGC GGTTTTGGAT ATTAGATACG GTGTTTCGAG AATTGCATAT AGTAAAGACT
601 TTGAAACTCT CAAAGTTGAT TTTCTTAGCA AGCTACCTGA AATGCTGAAA ATGTTCTGAAG
661 ATCGTTTATG TCATAAAACA TATTTAAATG GTGATCATGT AACCCATCCT GACTTCATGT
721 TGTATGACGC TCTTGATGTT GTTTTATACA TGGACCCAAT GTGCCTGGAT GCGTTCCTCA
781 AATTAGTTTG TTTTAAAAAA CGTATTGAAG CTATCCACAA AATTGATAAG TACTTGAAAT
841 CCAGCAAGTA TATAGCATGG CCTTTGCAGG GCTGGCAAGC CACGTTTGGT GGTGGCGACC
901 ATCCTCCAAA ATCGGATCTG ATCGAAGGTC GTGGGATCCC CAGGAATTCC CAGGTGCACA
961 GCTTCATTCG CTCGGCGCGC GAGCGCGAGA AGCACTCCGC CTGCTGCGTG CGCAACGACA
1021 GGTCGGGCTG CGTGCAGACC TCGGAGGAGG AGTGCTCGTC CACGCTGGCA GTGTGGGTGA
1081 AGTGGCCCAT CCATCCCAGC GCCCAGAGC TTGCGGGCCA CAAGAGACAG TTTGGCTCTG
1141 TCTGCCACCA GGATCCCAGG GTGTGTGATG AGCCCTCCTC CGAAGACCCT CATGAGTGGC
1201 CAGAAGACAT CACCAAGTGG CCGATCTGCA CCAAAAACAG CGCTGGGAAC CACACCAACC
1261 ATCCCCACAT GGAAGTGTG ATCACAGGAC GGCCCTGCTG CATTGGCACC AAGGGCAGGT
1321 GTGAGATCAC CTCCCGGGAG TACTGTGACT TCATGAGGGG CTAATTCCAT GAGGAGGCCA
1381 CGCTCTGCTC TCAGGTGCAC TGCATGGATG ATGTGTGTGG GCTCCTGCCT TTTCTCAACC
1441 CCGAGGTGCC TGACCAGTTC TACCGCCTGT GGCTATCCCT CTTCTGCAC GCCGGGATCT
1501 TGCACTGCCT GGTGTCCATC TGCTTCCAGA TGACTGTCCT GCGGGACCTG GAGAAGCTGG
1561 CAGGCTGGCA CCGCATAGCC ATCATCTACC TGCTGAGTGG TGTCACCGGC AACCTGGCCA
1621 GTGCCATCTT CCTGCCATAC CGAGCAGAGG TGGGTCCTGC TGGCTCCCAG TTCGGCATCC
1681 TGGCCTGCCT CTTCTGAGG CTCTTCCAGA GCTGGCAGAT CCTGGCGCGG CCCTGGCGTG
1741 CCTTCTTCAA GCTGCTGGCT GTGGTGCTCT TCCTCTTAC CTTTGGGCTG CTGCCGTGGA
1801 TTGACAACTT TGCCCACATC TCGGGGTTCA TCAGTGGCCT CTTCTCTCC TTCGCCTTCT
1861 TGCCCTACAT CAGCTTTGGC AAGTTTCGACC TGTACCGGAA ACGCTGCCAG ATCATCATCT
1921 TTCAGGTGGT CTTCTGGGC CTCCTGGCTG GCCTGGTGGT CCTCTTCTAC GTCTATCCTG
1981 TCCGCTGTGA GTGGTGTGAG TTCCTACCT GCATCCCCTT CACTGACAAG TTCTGTGAGA
2041 AGTACGAACT GGACGCTCAG CTCCACTGAG TCGACTCGAG CGGCCGCATC GTGACTGACT
2101 GACGATCTGC CTCGCGCGTT TCGGTGATGA CGGTGAAAC CTCTGACACA TGCAGCTCCC
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FIG. 29

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2161 GGAGACGGTC ACAGCTTGTC TGTAAGCGGA TGCCGGGAGC AGACAAGCCC GTCAGGGCGC
2221 GTCAGCGGGT GTTGGCGGGT GTCGGGGCGC AGCCATGACC CAGTCACGTA GCGATAGCGG
2281 AGTGTATAAT TCTTGAAGAC GAAAGGGCCT CGTGATACGC CTATTTTTAT AGGTTAATGT
2341 CATGATAATA ATGGTTTCTT AGACGTCAGG TGGCACTTTT CGGGGAAATG TGCGCGGAAC
2401 CCCTATTTGT TTATTTTTCT AAATACATTC AAATATGTAT CCGCTCATGA GACAATAACC
2461 CTGATAAATG CTTCAATAAT ATTGAAAAAG GAAGAGTATG AGTATTCAAC ATTTCCGTGT
2521 CGCCCTTATT CCCTTTTTTG CGGCATTTTG CTTTCTGT TTTGCTCACC CAGAAACGCT
2581 GGTGAAAGTA AAAGATGCTG AAGATCAGTT GGGTGCACGA GTGGGTACA TCGAACTGGA
2641 TCTCAACAGC GGTAAGATCC TTGAGAGTTT TCGCCCCGAA GAACGTTTTT CAATGATGAG
2701 CACTTTTAAA GTTCTGCTAT GTGGCGCGGT ATTATCCCGT GTTGACGCCG GGCAAGAGCA
2761 ACTCGGTCGC CGCATACACT ATTCTCAGAA TGACTTGGTT GAGTACTCAC CAGTCACAGA
2821 AAAGCATCTT ACGGATGGCA TGACAGTAAG AGAATTATGC AGTGCTGCCA TAACCATGAG
2881 TGATAACACT GCGGCCAACT TACTTCTGAC AACGATCGGA GGACCGAAGG AGCTAACCGC
2941 TTTTTTGAC AACATGGGGG ATCATGTAAC TCGCCTTGAT CGTTGGGAAC CGGAGCTGAA
3001 TGAAGCCATA CCAAACGACG AGCGTGACAC CACGATGCCT GCAGCAATGG CAACAACGTT
3061 GCGCAAATA TTAAGTGGCG AACTACTTAC TCTAGCTTCC CGGCAACAAT TAATAGACTG
3121 GATGGAGGCG GATAAAGTTG CAGGACCACT TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT
3181 TATTGCTGAT AAATCTGGAG CCGGTGAGCG TGGGTCTCGC GGTATCATTG CAGCACTGGG
3241 GCCAGATGGT AAGCCCTCCC GTATCGTAGT TATCTACACG ACGGGGAGTC AGGCAACTAT
3301 GGATGAACGA AATAGACAGA TCGCTGAGAT AGGTGCCTCA CTGATTAAGC ATTGGTAACT
3361 GTCAGACCAA GTTACTCAT ATATACTTTA GATTGATTTA AAACCTCATT TTTAATTTAA
3421 AAGGATCTAG GTGAAGATCC TTTTGTATA TCTCATGACC AAAATCCCTT AACGTGAGTT
3481 TTCGTTCCAC TGAGCGTCAG ACCCGTAGA AAAGATCAA GGATCTTCTT GAGATCCTTT
3541 TTTTCTGCGC GTAATCTGCT GCTTGCAAAC AAAAAACCA CCGCTACCAG CGGTGGTTTG
3601 TTTGCCGGAT CAAGAGCTAC CAACTCTTTT TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA
3661 GATACCAAAT ACTGTCCTTC TAGTGTAGCC GTAGTTAGGC CACCACTTCA AGAACTCTGT
3721 AGCACCGCCT ACATACCTCG CTCTGCTAAT CCTGTTACCA GTGGCTGCTG CCAGTGGCGA
3781 TAAGTCGTGT CTTACCGGGT TGGACTCAAG ACGATAGTTA CCGGATAAGG CGCAGCGGTC
3841 GGGCTGAACG GGGGGTTCGT GCACACAGCC CAGCTTGGAG CGAACGACCT ACACCGAACT
3901 GAGATACCTA CAGCGTGAGC TATGAGAAAG CGCCACGCTT CCCGAAGGGA GAAAGGCGGA
3961 CAGGTATCCG GTAAGCGGCA GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG
4021 AAACGCCTGG TATCTTTATA GTCCTGTCGG GTTTCGCCAC CTCTGACTTG AGCGTCGATT
4081 TTTGTGATGC TCGTCAGGGG GCGGAGCCT ATGGAAAAAC GCCAGCAACG CGGCCTTTTT
4141 ACGGTTCTTG GCCTTTTGCT GGCCTTTTGC TCACATGTTT TTTCTGCGT TATCCCCTGA
4201 TTCTGTGGAT AACCGTATTA CCGCCTTTGA GTGAGCTGAT ACCGCTCGCC GCAGCCGAAC
4261 GACCGAGCGC AGCGAGTCAG TGAGCGAGGA AGCGGAAGAG CGCCTGATGC GGTATTTTCT
4321 CCTTACGCAT CTGTGCGGTA TTTCACACCG CATAAATTCC GACACCATCG AATGGTGCAA

FIG. 29 (continued)

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4381 AACCTTTCGC GGTATGGCAT GATAGCGCCC GGAAGAGAGT CAATTCAGGG TGGTGAATGT
4441 GAAACCAGTA ACGTTATACG ATGTCGCAGA GTATGCCGGT GTCTCTTATC AGACCGTTTC
4501 CCGCGTGGTG AACCAGGCCA GCCACGTTTC TGCGAAAACG CGGGAAAAAG TGG AAGCGGC
4561 GATGGCGGAG CTGAATTACA TTCCCAACCG CGTGGCACAA CAACTGGCGG GCAAACAGTC
4621 GTTGCTGATT GGCGTTGCCA CCTCCAGTCT GGCCCTGCAC GCGCCGTCGC AAATTGTTCG
4681 GGCGATTAAA TCTCGCGCCG ATCAACTGGG TGCCAGCGTG GTGGTGTCTGA TGGTAGAACG
4741 AAGCGGCGTC GAAGCCTGTA AAGCGGCGGT GCACAATCTT CTCGCGCAAC GCGTCAGTGG
4801 GCTGATCATT AACTATCCGC TGGATGACCA GGATGCCATT GCTGTGGAAG CTGCCTGCAC
4861 TAATGTTCCG GCGTTATTTT TTGATGTCTC TGACCAGACA CCCATCAACA GTATTATTTT
4921 CTCCCATGAA GACGGTACGC GACTGGGCGT GGAGCATCTG GTCGCATTGG GTCACCAGCA
4981 AATCGCGCTG TTAGCGGGCC CATTAAAGTC TGTCTCGGCG CGTCTGCGTC TGGCTGGCTG
5041 GCATAAATAT CTCACTCGCA ATCAAATTCA GCCGATAGCG GAACGGGAAG GCGACTGGAG
5101 TGCCATGTCC GGTTTTCAAC AAACCATGCA AATGCTGAAT GAGGGCATCG TTCCCACTGC
5161 GATGCTGGTT GCCAACGATC AGATGGCGCT GGGCGCAATG CGCGCCATTA CCGAGTCCGG
5221 GCTGCGCGTT GGTGCGGATA TCTCGGTAGT GGGATACGAC GATACCGAAG ACAGCTCATG
5281 TTATATCCCG CCGTTAACCA CCATCAAACA GGATTTTCGC CTGCTGGGGC AAACCAGCGT
5341 GGACCGCTTG CTGCAACTCT CTCAGGGCCA GGCGGTGAAG GGCAATCAGC TGTTGCCCGT
5401 CTCACTGGTG AAAAGAAAAA CCACCCTGGC GCCCAATACG CAAACCGCCT CTCCCCGCGC
5461 GTTGGCCGAT TCATTAATGC AGCTGGCACG ACAGGTTTCC CGACTGGAAA GCGGGCAGTG
5521 AGCGCAACGC AATTAATGTG AGTTAGCTCA CTCATTAGGC ACCCCAGGCT TTACACTTTA
5581 TGCTTCCGGC TCGTATGTTG TGTGGAATTG TGAGCGGATA ACAATTTTAC ACAGGAAACA
5641 GCTATGACCA TGATTACGGA TTCACTGGCC GTCGTTTTAC AACGTCGTGA CTGGGAAAAC
5701 CCTGGCGTTA CCCAACTTAA TCGCCTTGCA GCACATCCCC CTTTCGCCAG CTGGCGTAAT
5761 AGCGAAGAGG CCCGCACCGA TCGCCCTTCC CAACAGTTGC GCAGCCTGAA TGGCGAATGG
5821 CGCTTTGCCT GGTTTCCGGC ACCAGAAGCG GTGCCGAAA GCTGGCTGGA GTGCGATCTT
5881 CCTGAGGCCG ATACTGTCGT CGTCCCCTCA AACTGGCAGA TGCACGGTTA CGATGCGCCC
5941 ATCTACACCA ACGTAACCTA TCCCATACG GTCAATCCGC CGTTTGTTC CACGGAGAAT
6001 CCGACGGGTT GTTACTCGCT CACATTTAAT GTTGATGAAA GCTGGCTACA GGAAGGCCAG
6061 ACGCGAATTA TTTTGTATGG CGTTGGAATT

FIG. 29 (continued)

34/62

(SEQ ID NO:66) ICT1024 coding region: 310-2879

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1  TAATACGACT CACTATAGGG GAATTGTGAG CGGATAACAA TTCCCCTCTA GACTTACAAT
61  TTCCATTTCG CATTCAAGGCT GCGCAACTGT TGGGAAGGGC GATCGGTACG GGCCTCTTCG
121 CTATTACGCC AGCTTGCGAA CGGTGGGTGC GCTGCAAGGC GATTAAGTTG GGTAACGCCA
181 GGATTCTCCC AGTCACGACG TTGTAAAACG ACGGCCAGCG AGAGATCTTG ATTGGCTAGC
241 AGAATAATTT TGTTTAACTT TAAGAAGGAG ATATACCATG GCGATATCCC GGGAGCTCGT
301 GGATCCGAAT TCCATGAGTG AGGCCCAGCAG GGACAGCAGC AGCAGCCTGC AGCGCAAGAA
361 GCCACCCTGG CTAAAGCTGG ACATTCCCTC TGCGGTGCCC CTGACGGCAG AAGAGCCCAG
421 CTTCCTGCAG CCCCTGAGGC GACAGGCTTT CCTGAGGAGT GTGAGTATGC CAGCCGAGAC
481 AGCCACATC TCTTCACCCC ACCATGAGCT CCGGCGGCCG GTGCTGCAAC GCCAGACGTC
541 CATCACACAG ACCATCCGCA GGGGGACCGC CGACTGGTTT GGAGTGAGCA AGGACAGTGA
601 CAGACCCAG AAATGGCAGC GCAAGAGCAT CCGTCACTGC AGCCAGCGCT ACGGGAAGCT
661 GAAGCCCCAG GTCTCCGGG AGCTGGACCT GCCCAGCCAG GACAACGTGT CGCTGACCAG
721 CACCGAGACG CCACCCCCAC TCTACGTGGG GCCATGCCAG CTGGGCATGC AGAAGATCAT
781 AGACCCCTG GCCCGTGGCC GTGCCTTCCG TGTGGCAGAT GACACTGCGG AAGGCCTGAG
841 TGCCCCACAC ACTCCCGTCA CGCCGGGTGC TGCTCCCTC TGCTCCTTCT CCAGCTCCCG
901 CTCAGGTTTC CACCGGCTCC CGCGCGGCCG CAAGCGAGAG TCGGTGGCCA AGATGAGCTT
961 CCGGGCGGCC GCAGCGCTGA TGAAAGGCCG CTCCGTTAGG GATGGCACCT TTCGCCGGGC
1021 ACGGCGTCGA AGCTTCACTC CAGCTAGCTT TCTGGAGGAG GACACAACTG ATTTCCCCGA
1081 TGAGCTGGAC ACATCCTTCT TTGCCCCGGA AGGTATCCTC CATGAAGAGC TGTCCACATA
1141 CCCGGATGAA GTTTTCGAGT CCCCATCGGA GGCAGCGCTA AAGGACTGGG AGAAGGCACC
1201 GGAGCAGGCG GACCTCACCG GCGGGGCCCT GGACCGCAGC GAGCTTGAGC GCAGCCACCT
1261 GATGCTGCCC TTGGAGCGAG GCTGGCGGAA GCAGAAGGAG GGCGCCGAGC CCCCAGGCC
1321 CAAGGTGCGG CTCCGACAGG AGGTGGTGAG CACCGCGGGG CCGCGACGGG GCCAGCGTAT
1381 CGCGGTGCCG GTGCGCAAGC TCTTCGCCCC GGAGAAGCGG CCGTATGGGC TGGGCATGGT
1441 GGGACGGCTC ACCAACCACA CCTACCGCAA GCGCATCGAC AGCTTCGTCA AGCGCCAGAT
1501 CGAGGACATG GACGACCACA GGCCCTTCTT CACCTACTGG CTTACCTTCG TGCACTCGCT
1561 CGTCACCATC CTAGCCGTGT GCATCTATGG CATCGCGCCC GTGGGCTTCT CGCAGCATGA
1621 GACGGTGGAC TCGGTGCTGC GGAACCGCGG GGTCTACGAG AACGTCAAGT ACGTGCAGCA
1681 GGAGAACTTC TGGATCGGGC CCAGCTCGGA GGCCCTCATC CACCTGGGCG CCAAGTTTTC
1741 GCCCTGCATG CGCCAGGACC CGCAGGTGCA CAGCTTCATT CGCTCGGCGC GCGAGCGCGA
1801 GAAGCACTCC GCCTGCTGCG TGCGCAACGA CAGGTGCGGC TGCCTGCAGA CCTCGGAGGA
1861 GGAGTGCTCG TCCACGCTGG CAGTGTGGGT GAAGTGGCCC ATCCATCCCA GCGCCCCAGA
1921 GCTTGCGGGC CACAAGAGAC AGTTTGGCTC TGTCTGCCAC CAGGATCCCA GGGTGTGTGA
1981 TGAGCCCTCC TCCGAAGACC CTCATGAGTG GCCAGAAGAC ATCACCAAGT GGCCGATCTG
2041 CACCAAAAAC AGCGCTGGGA ACCACACCAA CCATCCCCAC ATGGACTGTG TCATCACAGG
2101 ACGGCCCTGC TGCATTGGCA CCAAGGGCAG GTGTGAGATC ACCTCCCGGG AGTACTGTGA
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FIG. 30

35/62

2161 CTTTCATGAGG GGCTACTTCC ATGAGGAGGC CACGCTCTGC TCTCAGGTGC ACTGCATGGA
 2221 TGATGTGTGT GGGCTCCTGC CTTTTCTCAA CCCCAGAGTG CCTGACCAGT TCTACCGCCT
 2281 GTGGCTATCC CTCTTCCTGC ACGCCGGGAT CTTGCACTGC CTGGTGTCCA TCTGCTTCCA
 2341 GATGACTGTC CTGCGGGACC TGGAGAAGCT GGCAGGCTGG CACCGCATAG CCATCATCTA
 2401 CCTGCTGAGT GGTGTCACCG GCAACCTGGC CAGTGCCATC TTCCTGCCAT ACCGAGCAGA
 2461 GGTGGGTCCT GCTGGCTCCC AGTTCGGCAT CCTGGCCTGC CTCTTCGTGG AGCTCTTCCA
 2521 GAGCTGGCAG ATCCTGGCGC GGCCCTGGCG TGCCTTCTTC AAGCTGCTGG CTGTGGTGTCT
 2581 CTTCTCTTTC ACCTTTGGGC TGCTGCCGTG GATTGACAAC TTTGCCACACA TCTCGGGGTT
 2641 CATCAGTGGC CTCTTCCTCT CCTTCGCCTT CTTGCCCTAC ATCAGCTTTG GCAAGTTCGA
 2701 CCTGTACCGG AAACGCTGCC AGATCATCAT CTTTCAGGTG GTCTTCCTGG GCCTCCTGGC
 2761 TGGCCTGGTG GTCCTCTTCT ACGTCTATCC TGTCCGCTGT GAGTGGTGTG AGTTCCTCAC
 2821 CTGCATCCCC TTCCTGACA AGTTCCTGTGA GAAGTACGAA CTGGACGCTC AGCTCCACAT
 2881 CGATACGCGT TCGAAGCTTG CGGCCGCACA GCTGTATACA CGTGCAAGCC AGCCAGAACT
 2941 CGCTCCTGAA GACCCAGAGG ATCTCGAGCA CCACCACCAC CACCACTAAT GTTAATTAAG
 3001 TTGGGCGTTG TAATCATAGT CATAATCAAT ACTCCTGACT GCGTTAGCAA TTAACTGTG
 3061 ATAACTACC GCATTAAAGC TATTCGATGA TAAGCTGTCA AACATGATAA TTCTTGAAGA
 3121 CGAAAGGGCC TAGGCTGATA AAACAGAATT TGCCTGGCGG CAGTAGCGCG GTGGTCCCAC
 3181 CTGACCCCAT GCCGAACCTCA GAAGTGAAAC GCCGTAGCGC CGATGGTAGT GTGGGGTCTC
 3241 CCCATGCGAG AGTAGGGAAC TGCCAGGCAT CAAATAAAAC GAAAGGCTCA GTCGAAAGAC
 3301 TGGGCCTTTC GTTTTATCTG TTGTTTGTGCG GTGAACGCTC TCCTGAGTAG GACAAATCCG
 3361 CCGGGAGCGG ATTTGAACGT TGCGAAGCAA CGGCCCGGAG GGTGGCGGGC AGGACGCCCCG
 3421 CCATAAACTG CCAGGCATCA AATTAAGCAG AAGGCCATCC TGACGGATGG CCTTTTTGCG
 3481 TTTCTACAAA CTCTTTTGTG TATTTTTCTA AATACATTCA AATATGTATC CGCTGAGCAA
 3541 TAACTAGCAT AACCCTTGG GGCCTCTAAA CGGGTCTTGA GGGGTTTTTT GCTGAAAGGA
 3601 GGAACATAT CCGGATTGGC GAATGGGACG CGCCCTGTAG CGGCGCATTA AGCGCGGCGG
 3661 GTGTGGTGGT TACGCGCAGC GTGACCGCTA CACTTGCCAG CGCCCTAGCG CCCGCTCCTT
 3721 TCGCTTTCTT CCCTTCCTTT CTCGCCACGT TCGCCGGCTT TCCCCGTCAA GCTCTAAATC
 3781 GGGGGCTCCC TTTAGGGTTC CGATTTAGTG CTTTACGGCA CCTCGACCCC AAAAACTTG
 3841 ATTAGGGTGA TGGTTCACGT AGTGGGCCAT CGCCCTGATA GACGGTTTTT CGCCCTTTGA
 3901 CGTTGGAGTC CACGTTCTTT AATAGTGGAC TCTTGTTCCA AACTGGAACA AACTCAACC
 3961 CTATCTCGGT CTATTCTTTT GATTTATAAG GGATTTTGCC GATTCGGCC TATTGGTTAA
 4021 AAAATGAGCT GATTTAACA AAATTTAACG CGAATTTTAA CAAAATATTA ACGTTTACAA
 4081 TTTCTGGCGG CACGATGGCA TGAGATTATC AAAAAGGATC TTCACCTAGA TCCTTTTAAA
 4141 TTAAAAATGA AGTTTTAAAT CAATCTAAAG TATATATGAG TAAACTTGGT CTGACAGTTA
 4201 CCAATGCTTA ATCAGTGAGG CACCTATCTC AGCGATCTGT CTATTTCTGT CATCCATAGT
 4261 TGCCTGACTC CCCGTCGTGT AGATAACTAC GATACGGGAG GGCTTACCAT CTGGCCCCAG
 4321 TGCTGCAATG ATACCGCGAG ACCCACGCTC ACCGGCTCCA GATTTATCAG CAATAAACCA

FIG. 30 (continued)

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4381 GCCAGCCGGA AGGGCCGAGC GCAGAAGTGG TCCTGCAACT TTATCCGCCT CCATCCAGTC
4441 TATTAATTGT TGCCGGGAAG CTAGAGTAAG TAGTTCGCCA GTTAATAGTT TGC GCAACGT
4501 TGTTGCCATT GCTACAGGCA TCGTGGTGTC ACGCTCGTCG TTTGGTATGG CTTCATTCAG
4561 CTCCGGTTCC CAACGATCAA GGCGAGTTAC ATGATCCCCC ATGTTGTGCA AAAAAGCGGT
4621 TAGCTCCTTC GGTCCTCCGA TCGTTGTCAG AAGTAAGTTG GCCGCAGTGT TATCACTCAT
4681 GGTTATGGCA GCACTGCATA ATTCTCTTAC TGTCATGCCA TCCGTAAGAT GCTTTTCTGT
4741 GACTGGTGAG TACTCAACCA AGTCATTCTG AGAATAGTGT ATGCGGCGAC CGAGTTGCTC
4801 TTGCCCCGGC TCAATACGGG ATAATACCGC GCCACATAGC AGAACTTTAA AAGTGCTCAT
4861 CATTGGAAAA CGTTCTTCGG GGCGAAAAC CTCAAGGATC TTACCGCTGT TGAGATCCAG
4921 TTCGATGTAA CCCACTCGTG CACCCAAC TG ATCTTCAGCA TCTTTTACTT TCACCAGCGT
4981 TTCTGGGTGA GCAAAAACAG GAAGGCAAAA TGCCGCAAAA AAGGGAATAA GGGCGACACG
5041 GAAATGTTGA ATACTCATAC TCTTCCTTTT TCAATCATGA CCAAAATCCC TTAACGTGAG
5101 TTTTCGTTCC ACTGAGCGTC AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT
5161 TTTTTTCTGC GCGTAATCTG CTGCTTGCAA ACAAAAAAAC CACCGCTACC AGCGGTGGTT
5221 TGTTTGCCGG ATCAAGAGCT ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG
5281 CAGATACCAA ATACTGTCCT TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT
5341 GTAGCACCGC CTACATACCT CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC
5401 GATAAGTCGT GTCTTACCGG GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG
5461 TCGGGCTGAA CGGGGGGTTC GTGCACACAG CCCAGCTTGG AGCGAACGAC CTACACCGAA
5521 CTGAGATACC TACAGCGTGA GCTATGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG
5581 GACAGGTATC CGGTAAGCGG CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG
5641 GGAAACGCCT GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA
5701 TTTTGTGAT GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA CGCGGCCTTT
5761 TTACGGTTCC TGGCCTTTTG CTGGCCTTTT GCTCACATGT TCTTTCCTGC GTTATCCCCT
5821 GATTCTGTGG ATAACCGTAT TACCGCCTTT GAGTGAGCTG ATACCGCTCG CCGCAGCCGA
5881 ACGACCGAGC GCAGCGAGTC AGTGAGCGAG GAAGCCGGCG ATAATGGCCT GCTTCTCGCC
5941 GAAACGTTTG GTGGCGGGAC CAGTGACGAA GGCTTGAGCG AGGGCGTGCA AGATTCCGAA
6001 TACCGCAAGC GACAGGCCGA TCATCGTCGC GCTCCAGCGA AAGCGGTCCT CGCCGAAAAT
6061 GACCCAGAGC GCTGCCGGCA CCTGTCCTAC GAGTTGCATG ATAAAGAAGA CAGTCATAAG
6121 TGCGGCGACG ACCGGTGAAT TGTGAGCGCT CACAATTCTC GTGACATCAT AACGTCCCGC
6181 GAAAT

FIG. 30 (continued)

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(SEQ ID NO:68) Coding region for the N terminus 400 aa of
ICT1024: 314-1515

```
1  TAATACGACT CACTATAGGG GAATTGTGAG CGGATAACAA TTCCCCTCTA GACTTACAAT
61  TTCCATTTCGC CATTCAAGGCT GCGCAACTGT TGGGAAGGGC GATCGGTACG GGCCTCTTCG
121 CTATTACGCC AGCTTGCGAA CGGTGGGTGC GCTGCAAGGC GATTAAGTTG GGTAACGCCA
181 GGATTCTCCC AGTCACGACG TTGTAAAACG ACGGCCAGCG AGAGATCTTG ATTGGCTAGC
241 AGAATAATTT TGTTTAACTT TAAGAAGGAG ATATACCATG GCGATATCCC GGGAGCTCGT
301 GGATCCGAAT TCCATGAGTG AGGCCCGCAG GGACAGCACG AGCAGCCTGC AGCGCAAGAA
361 GCCACCCTGG CTAAAGCTGG ACATTCCCTC TGCGGTGCCC CTGACGGCAG AAGAGCCCAG
421 CTTCCCTGCAG CCCCTGAGGC GACAGGCTTT CCTGAGGAGT GTGAGTATGC CAGCCGAGAC
481 AGCCCACATC TCTTCACCCC ACCATGAGCT CCGGCGGCCG GTGCTGCAAC GCCAGACGTC
541 CATCACACAG ACCATCCGCA GGGGGACCGC CGACTGGTTT GGAGTGAGCA AGGACAGTGA
601 CAGCACCCAG AAATGGCAGC GCAAGAGCAT CCGTCACTGC AGCCAGCGCT ACGGGAAGCT
661 GAAGCCCCAG GTCCCTCCGGG AGCTGGACCT GCCCAGCCAG GACAACGTGT CGCTGACCAG
721 CACCGAGACG CCACCCCCAC TCTACGTGGG GCCATGCCAG CTGGGCATGC AGAAGATCAT
781 AGACCCCCTG GCCCCGTGGCC GTGCCTTCCG TGTGGCAGAT GACACTGCGG AAGGCCTGAG
841 TGCCCCACAC ACTCCCGTCA CGCCGGGTGC TGCCTCCCTC TGCTCCTTCT CCAGCTCCCG
901 CTCAGGTTTC CACCGGCTCC CGCGGCGGCG CAAGCGAGAG TCGGTGGCCA AGATGAGCTT
961 CCGGGCGGCC GCAGCGCTGA TGAAAGGCCG CTCCGTTAGG GATGGCACCT TTCGCCGGGC
1021 ACGGCGTCGA AGCTTCACTC CAGCTAGCTT TCTGGAGGAG GACACAAC TG ATTTCCCCGA
1081 TGAGCTGGAC ACATCCTTCT TTGCCCCGGA AGGTATCCTC CATGAAGAGC TGTCCACATA
1141 CCCGGATGAA GTTTTCGAGT CCCCATCGGA GGCAGCGCTA AAGGACTGGG AGAAGGCACC
1201 GGAGCAGGCG GACCTCACCG GCGGGGCCCT GGACCGCAGC GAGCTTGAGC GCAGCCACCT
1261 GATGCTGCCC TTGGAGCGAG GCTGGCGGAA GCAGAAGGAG GGCGCCGAG CCCCGCAGCC
1321 CAAGGTGCGG CTCCGACAGG AGGTGGTGAG CACCGCGGGG CCGCGACGGG GCCAGCGTAT
1381 CGCGGTGCCG GTGCGCAAGC TCTTCGCCCG GGAGAAGCGG CCGTATGGGC TGGGCATGGT
1441 GGGACGGCTC ACCAACCGCA CCTACCGCAA GCGCATCGAC AGCTTCGTCA AGCGCCAGAT
1501 CGAGGACATG GACATCGATA CGCGTTCGAA GCTTGCGGCC GCACAGCTGT ATACACGTGC
1561 AAGCCAGCCA GAACTCGCTC CTGAAGACCC AGAGGATCTC GAGCACCACC ACCACCACCA
1621 CTAATGTAA TTAAGTTGGG CGTTGTAATC ATAGTCATAA TCAATACTCC TGA CTGCGTT
1681 AGCAATTTAA CTGTGATAAA CTACCGCATT AAAGCTATTC GATGATAAGC TGTCAAACAT
1741 GATAATTCTT GAAGACGAAA GGGCCTAGGC TGATAAAACA GAATTTGCCT GGCGGCAGTA
1801 GCGCGGTGGT CCCACCTGAC CCCATGCCGA ACTCAGAAGT GAAACGCCGT AGCGCCGATG
1861 GTAGTGTGGG GTCTCCCCAT GCGAGAGTAG GGAAGTGCCA GGCATCAAAT AAAACGAAAG
1921 GCTCAGTCGA AAGACTGGGC CTTTCGTTTT ATCTGTTGTT TGTCGGTGAA CGCTCTCCTG
1981 AGTAGGACAA ATCCGCCGGG AGCGGATTTG AACGTTGCCA AGCAACGGCC CGGAGGGTGG
2041 CGGGCAGGAC GCCCGCCATA AACTGCCAGG CATCAAATTA AGCAGAAGGC CATCCTGACG
```

FIG. 31

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2101 GATGGCCTTT TTGCGTTTCT ACAAACCTCTT TTGTTTATTT TTCTAAATAC ATTCAAATAT
2161 GTATCCGCTG AGCAATAACT AGCATAACCC CTTGGGGCCT CTAAACGGGT CTTGAGGGGT
2221 TTTTGTCTGA AAGGAGGAAC TATATCCGGA TTGGCGAATG GGACGCGCCC TGTAGCGGCG
2281 CATTAAGCGC GGCGGGTGTG GTGGTTACGC GCAGCGTGAC CGCTACACTT GCCAGCGCCC
2341 TAGCGCCCGC TCCTTTCGCT TTCTTCCCTT CCTTCTCGC CACGTTTCGCC GGCTTTCCCC
2401 GTCAAGCTCT AAATCGGGGG CTCCCTTTAG GGTTCCGATT TAGTGCTTTA CGGCACCTCG
2461 ACCCCAAAAA ACTTGATTAG GGTGATGGTT CACGTAGTGG GCCATCGCCC TGATAGACGG
2521 TTTTTCGCCC TTTGACGTTG GAGTCCACGT TCTTTAATAG TGGACTCTTG TTCCAAACTG
2581 GAACAACACT CAACCCTATC TCGGTCTATT CTTTGTATTT ATAAGGGATT TTGCCGATTT
2641 CGGCCTATTG GTTAAAAAAT GAGCTGATTT AACAAAAATT TAACGCGAAT TTAAACAAAA
2701 TATTAACGTT TACAATTTCT GGCGGCACGA TGGCATGAGA TTATCAAAAA GGATCTTCAC
2761 CTAGATCCTT TTAAATTAAA AATGAAGTTT TAAATCAATC TAAAGTATAT ATGAGTAAAC
2821 TTGGTCTGAC AGTTACCAAT GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATT
2881 TCGTTCATCC ATAGTTGCCT GACTCCCCGT CGTGTAGATA ACTACGATAC GGGAGGGCTT
2941 ACCATCTGGC CCCAGTGCTG CAATGATACC GCGAGACCCA CGCTCACC GG CTCCAGATTT
3001 ATCAGCAATA AACCAGCCAG CCGGAAGGGC CGAGCGCAGA AGTGGTCCCTG CAACTTTATC
3061 CGCCTCCATC CAGTCTATTA ATTGTTGCCG GGAAGCTAGA GTAAGTAGTT CGCCAGTTAA
3121 TAGTTTGCGC AACGTTGTTG CCATTGCTAC AGGCATCGTG GTGTCACGCT CGTCGTTTGG
3181 TATGGCTTCA TTCAGCTCCG GTTCCCAACG ATCAAGGCGA GTTACATGAT CCCCATGTT
3241 GTGCAAAAAA GCGGTTAGCT CCTTCGGTCC TCCGATCGTT GTCAGAAGTA AGTTGGCCGC
3301 AGTGTTATCA CTCATGGTTA TGGCAGCACT GCATAATTCT CTTACTGTCA TGCCATCCGT
3361 AAGATGCTTT TCTGTGACTG GTGAGTACTC AACCAAGTCA TTCTGAGAAT AGTGTATGCG
3421 GCGACCGAGT TGCTCTTGCC CGGCGTCAAT ACGGGATAAT ACCGCGCCAC ATAGCAGAAC
3481 TTTAAAAGTG CTCATCATTTG GAAAACGTTT TTCGGGGCGA AAACCTCTCA GGATCTTACC
3541 GCTGTTGAGA TCCAGTTCGA TGTAACCCAC TCGTGCACCC AACTGATCTT CAGCATCTTT
3601 TACTTTCACC AGCGTTTCTG GGTGAGCAAA AACAGGAAGG CAAAATGCCG CAAAAAGGG
3661 AATAAGGGCG ACACGGAAAT GTTGAATACT CATACTCTTC CTTTTTCAAT CATGACCAAA
3721 ATCCCTTAAC GTGAGTTTTT GTTCCACTGA GCGTCAGACC CCGTAGAAAA GATCAAAGGA
3781 TCTTCTTGAG ATCCTTTTTT TCTGCGCGTA ATCTGCTGCT TGCAAACAAA AAAACCACCG
3841 CTACCAGCGG TGGTTTGTTT GCCGGATCAA GAGCTACCAA CTCTTTTTCC GAAGGTAAC
3901 GGCTTCAGCA GAGCGCAGAT ACCAAATACT GTCCTTCTAG TGTAGCCGTA GTTAGGCCAC
3961 CACTTCAAGA ACTCTGTAGC ACCGCTACA TACCTCGCTC TGCTAATCCT GTTACCAGTG
4021 GCTGCTGCCA GTGGCGATAA GTCGTGTCTT ACCGGGTTGG ACTCAAGACG ATAGTTACCG
4081 GATAAGGCGC AGCGGTCGGG CTGAACGGGG GGTTCGTGCA CACAGCCCAG CTTGGAGCGA
4141 ACGACCTACA CCGAACTGAG ATACCTACAG CGTGAGCTAT GAGAAAGCGC CACGCTTCCC
4201 GAAGGGAGAA AGGCGGACAG GTATCCGGTA AGCGGCAGGG TCGGAACAGG AGAGCGCAGG
4261 AGGGAGCTTC CAGGGGGAAA CGCCTGGTAT CTTTATAGTC CTGTCGGGTT TCGCCACCTC

FIG. 31 (continued)

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4321 TGA CTTGAGC GTCGATTTTT GTGATGCTCG TCAGGGGGGC GGAGCCTATG GAAAAACGCC
4381 AGCAACGCGG CCTTTTTACG GTTCCTGGCC TTTTGCTGGC CTTTGCTCA CATGTTCTTT
4441 CCTGCGTTAT CCCCTGATTC TGTGGATAAC CGTATTACCG CCTTTGAGTG AGCTGATACC
4501 GCTCGCCGCA GCCGAACGAC CGAGCGCAGC GAGTCAGTGA GCGAGGAAGC CGGCGATAAT
4561 GGCCTGCTTC TCGCCGAAAC GTTTGGTGGC GGGACCAAGT ACGAAGGCTT GAGCGAGGGC
4621 GTGCAAGATT CCGAATACCG CAAGCGACAG GCCGATCATC GTCGCGCTCC AGCGAAAGCG
4681 GTCCTCGCCG AAAATGACCC AGAGCGCTGC CGGCACCTGT CCTACGAGTT GCATGATAAA
4741 GAAGACAGTC ATAAGTGCGG CGACGACCGG TGAATTGTGA GCGCTCACAA TTCTCGTGAC
4801 ATCATAACGT CCCGCGAAAT

FIG. 31 (continued)

40/62

(SEQ ID NO 69) Coding region for the C terminus 373 aa of
ICT1024: 308-1431

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1  TAATACGACT CACTATAGGG GAATTGTGAG CGGATAACAA TTCCCCTCTA GACTTACAAT
61  TTCCATTTCGC CATTTCAGGCT GCGCAACTGT TGGGAAGGGC GATCGGTACG GGCCTCTTCG
121 CTATTACGCC AGCTTGCGAA CGGTGGGTGC GCTGCAAGGC GATTAAGTTG GGTAACGCCA
181 GGATTCTCCC AGTCACGACG TTGTAAAACG ACGGCCAGCG AGAGATCTTG ATTTGGCTAGC
241 AGAATAATTT TGTTTAACTT TAAGAAGGAG ATATACCATG GCGATATCCC GGGAGCTCGT
301 GGATCCGAAT TCCCAGGTGC ACAGCTTCAT TCGCTCGGCG CGCGAGCGCG AGAAGCACTC
361 CGCCTGCTGC GTGCGCAACG ACAGGTGCGG CTGCGTGCAG ACCTCGGAGG AGGAGTGCTC
421 GTCCACGCTG GCAGTGTGGG TGAAGTGGCC CATCCATCCC AGCGCCCCAG AGCTTGCGGG
481 CCACAAGAGA CAGTTTGGCT CTGTCTGCCA CCAGGATCCC AGGGTGTGTG ATGAGCCCTC
541 CTCCGAAGAC CCTCATGAGT GGCCAGAAGA CATACCAAG TGGCCGATCT GCACCAAAAA
601 CAGCGCTGGG AACCACACCA ACCATCCCCA CATGGACTGT GTCATCACAG GACGGCCCTG
661 CTGCATTGGC ACCAAGGGCA GGTGTGAGAT CACCTCCCGG GAGTACTGTG ACTTCATGAG
721 GGGCTACTTC CATGAGGAGG CCACGCTCTG CTCTCAGGTG CACTGCATGG ATGATGTGTG
781 TGGGCTCCTG CCTTTTCTCA ACCCCGAGGT GCCTGACCAG TTCTACCGCC TGTGGCTATC
841 CCTCTTCCTG CACGCCGGGA TCTTGCACTG CCTGGTGTCC ATCTGCTTCC AGATGACTGT
901 CCTGCGGGAC CTGGAGAAGC TGGCAGGCTG GCACCGCATA GCCATCATCT ACCTGCTGAG
961 TGGTGTCAAC GGCAACCTGG CCAGTGCCAT CTTCTGCCA TACCGAGCAG AGGTGGGTCC
1021 TGCTGGCTCC CAGTTCGGCA TCCTGGCCTG CCTCTTCGTG GAGCTCTTCC AGAGCTGGCA
1081 GATCCTGGCG CGGCCCTGGC GTGCCTTCTT CAAGCTGCTG GCTGTGGTGC TCTTCTCTT
1141 CACCTTTGGG CTGCTGCCGT GGATTGACAA CTTTGCCAC ATCTCGGGT TCATCAGTGG
1201 CCTCTTCCTC TCCTTCGCCT TCTTGCCCTA CATCAGCTTT GGCAAGTTCG ACCTGTACCG
1261 GAAACGCTGC CAGATCATCA TCTTTCAGGT GGTCTTCCTG GGCCTCCTGG CTGGCCTGGT
1321 GGTCTCTTTC TACGTCTATC CTGTCCGCTG TGAGTGGTGT GAGTTCCTCA CCTGCATCCC
1381 CTTCACTGAC AAGTTCTGTG AGAAGTACGA ACTGGACGCT CAGCTCCACA TCGATACGCG
1441 TTCGAAGCTT GCGGCCGCAC AGCTGTATAC ACGTGCAAGC CAGCCAGAAC TCGCTCCTGA
1501 AGACCCAGAG GATCTCGAGC ACCACCACCA CCACCACTAA TGTTAATTAA GTTGGGCGTT
1561 GTAATCATAG TCATAATCAA TACTCCTGAC TGCGTTAGCA ATTTAACTGT GATAAACTAC
1621 CGCATTAAG CTATTCGATG ATAAGCTGTC AAACATGATA ATTCTTGAAG ACGAAAGGGC
1681 CTAGGCTGAT AAAACAGAAAT TTGCCTGGCG GCAGTAGCGC GGTGGTCCCA CCTGACCCCA
1741 TGCCGAAGTC AGAAGTGAAA CGCCGTAGCG CCGATGGTAG TGTGGGGTCT CCCCATGCGA
1801 GAGTAGGGAA CTGCCAGGCA TCAAATAAAA CGAAAGGCTC AGTCGAAAGA CTGGGCCTTT
1861 CGTTTTATCT GTTGTGTTGTC GGTGAACGCT CTCCTGAGTA GGACAAATCC GCCGGGAGCG
1921 GATTTGAACG TTGCGAAGCA ACGGCCCGGA GGGTGGCGGG CAGGACGCCC GCCATAAACT
1981 GCCAGGCATC AAATTAAGCA GAAGGCCATC CTGACGGATG GCCTTTTTTG GTTTCTACAA
2041 ACTCTTTTGT TTATTTTCT AAATACATTC AAATATGTAT CCGCTGAGCA ATAAC TAGCA
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FIG. 32

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2101 TAACCCCTTG GGGCCTCTAA ACGGGTCTTG AGGGGTTTTT TGCTGAAAGG AGGAACTATA
2161 TCCGGATTGG CGAATGGGAC GCGCCCTGTA GCGGCGCATT AAGCGCGGCG GGTGTGGTGG
2221 TTACGCGCAG CGTGACCGCT ACACTTGCCA GCGCCCTAGC GCCCGCTCCT TTCGCTTTCT
2281 TCCCTTCCTT TCTCGCCACG TTCGCCGGCT TTCCCCGTCA AGCTCTAAAT CGGGGGCTCC
2341 CTTTAGGGTT CCGATTAGT GCTTTACGGC ACCTCGACCC CAAAAAAGTT GATTAGGGTG
2401 ATGGTTCACG TAGTGGGCCA TCGCCCTGAT AGACGGTTTT TCGCCCTTTG ACGTTGGAGT
2461 CCACGTTCTT TAATAGTGGA CTCTTGTTCC AAAGTGAAC AACACTCAAC CCTATCTCGG
2521 TCTATTCTTT TGATTTATAA GGGATTTTGC CGATTTTCGGC CTATTGGTTA AAAAATGAGC
2581 TGATTTAACA AAAATTTAAC GCGAATTTTA ACAAATATT AACGTTTACA ATTTCTGGCG
2641 GCACGATGGC ATGAGATTAT CAAAAAGGAT CTTACCTAG ATCCTTTTAA ATTAAAAATG
2701 AAGTTTTAAA TCAATCTAAA GTATATATGA GTAACTTGG TCTGACAGTT ACCAATGCTT
2761 AATCAGTGAG GCACCTATCT CAGCGATCTG TCTATTTTCG TCATCCATAG TTGCCTGACT
2821 CCCCCTCGTG TAGATAACTA CGATACGGGA GGGCTTACCA TCTGGCCCCA GTGCTGCAAT
2881 GATACGCGA GACCCACGCT CACCGGCTCC AGATTTATCA GCAATAAACC AGCCAGCCGG
2941 AAGGGCCGAG CGCAGAAGTG GTCCTGCAAC TTTATCCGCC TCCATCCAGT CTATTAATTG
3001 TTGCCGGGAA GCTAGAGTAA GTAGTTCGCC AGTTAATAGT TTGCGCAACG TTGTTGCCAT
3061 TGCTACAGGC ATCGTGGTGT CACGCTCGTC GTTGGTATG GCTTCATTCA GCTCCGGTTC
3121 CCAACGATCA AGGCGAGTTA CATGATCCCC CATGTTGTGC AAAAAAGCGG TTAGCTCCTT
3181 CGGTCCTCCG ATCGTTGTCA GAAGTAAGTT GGCCGCAGTG TTATCACTCA TGGTTATGGC
3241 AGCACTGCAT AATTCTCTTA CTGTCATGCC ATCCGTAAGA TGCTTTTCTG TGAAGGTGA
3301 GTACTCAACC AAGTCATTCT GAGAATAGTG TATGCGGCGA CCGAGTTGCT CTTGCCCCGGC
3361 GTCAATACGG GATAATACCG CGCCACATAG CAGAACTTTA AAAGTGCTCA TCATTGGAAA
3421 ACGTTCTTCG GGGCGAAAAC TCTCAAGGAT CTTACCGCTG TTGAGATCCA GTTCGATGTA
3481 ACCCACTCGT GCACCAACT GATCTTCAGC ATCTTTTACT TTCACCAGCG TTTCTGGGTG
3541 AGCAAAAACA GGAAGGCAA ATGCCGCAA AAAGGGAATA AGGGCGACAC GGAAATGTTG
3601 AATACTCATA CTCTTCCTTT TTCAATCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTT
3661 CACTGAGCGT CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGATCC TTTTTTTCTG
3721 CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTGCCG
3781 GATCAAGAGC TACCAACTCT TTTTCCGAAG GTAAGTGGCT TCAGCAGAGC GCAGATACCA
3841 AATACTGTCC TTCTAGTGTA GCCGTAGTTA GGCCACCACT TCAAGAACTC TGTAGCACCG
3901 CCTACATACC TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG
3961 TGTCTTACCG GGTTGGACTC AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA
4021 ACGGGGGGTT CGTGCACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC
4081 CTACAGCGTG AGCTATGAGA AAGCGCCACG CTTCCCGAAG GGAGAAAGGC GGACAGGTAT
4141 CCGGTAAGCG GCAGGGTCGG AACAGGAGAG CGCACGAGGG AGCTTCCAGG GGGAAACGCC
4201 TGGTATCTTT ATAGTCCTGT CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTTGTGA
4261 TGCTCGTCAG GGGGCGGAG CCTATGGAAA AACGCCAGCA ACGCGGCCTT TTTACGGTTC

FIG. 32 (continued)

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4321 CTGGCCTTTT GCTGGCCTTT TGCTCACATG TTCTTTCCTG CGTTATCCCC TGATTCTGTG
4381 GATAACCGTA TTACCGCCTT TGAGTGAGCT GATACCGCTC GCCGCAGCCG AACGACCGAG
4441 CGCAGCGAGT CAGTGAGCGA GGAAGCCGGC GATAATGGCC TGCTTCTCGC CGAAACGTTT
4501 GGTGGCGGGA CCAGTGACGA AGGCTTGAGC GAGGGCGTGC AAGATTCCGA ATACCGCAAG
4561 CGACAGGCCG ATCATCGTCG CGCTCCAGCG AAAGCGGTCC TCGCCGAAAA TGACCCAGAG
4621 CGCTGCCGGC ACCTGTCCTA CGAGTTGCAT GATAAAGAAG ACAGTCATAA GTGCGGCGAC
4681 GACCGGTGAA TTGTGAGCGC TCACAATTCT CGTGACATCA TAACGTCCCG CGAAAT

FIG. 32 (continued)

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mRNA sequence
NM_003299
Tumor rejection antigen 1
Gp96
2780 bp
Coding Regions: 106 - 2517

```
gtggcgccgac cgcgcggctg gaggtgtgag gatccgaacc caggggtggg ggggtggaggc 61
ggctcctgcg atcgaagggg acttgagact caccggccgc acgccatgag ggcctgtgg 121
gtgctgggcc tctgctgcgt cctgctgacc ttcgggtcgg tcagagctga cgtgaagtt 181
gatgtggatg gtacagtaga agaggatctg ggtaaaagta gagaaggatc aaggacggat 241
gatgaagtag tacagagaga ggaagaagct attcagttgg atggattaaa tgcatacaca 301
ataagagaac tttagagaga gtcggaagaa tttgccttcc aagccgaagt taacagaatg 361
atgaaactta tcatcaattc attgtataaa aataaagaga ttttctgag agaactgatt 421
tcaaagtctt ctgatgcttt agataagata aggctaatat cactgactga tgaatgct 481
ctttctggaa atgaggaact aacagtcaaa attaagtgtg ataaggagaa gaacctgctg 541
catgtcacag acaccggtgt aggaatgacc agagaagagt tggtaaaaa ccttggtacc 601
atagccaaat ctgggacaag cgagttttta aacaaaatga ctgaagcaca ggaagatggc 661
cagtcaactt ctgaattgat tggccagttt ggtgtcgggt tctattccgc ctctctgtga 721
gcagataagg ttattgtcac ttcaaacac aacaacgata cccagcacat ctgggagctc 781
gactccaatg aattttctgt aattgctgac ccaagaggaa aactctagg acggggaacg 841
acaattacce ttgtcttaaa agaagaagca tctgattacc ttgaattgga tacaattaaa 901
aatctcgta aaaaatattc acagttcata aactttccta tttatgtatg gacgagcaag 961
actgaaactg ttgaggagcc catggaggaa gaagaagcag ccaaagaaga gaaagaagaa 1021
tctgatgatg aagctgcagt agaggaagaa gaagaagaaa agaaccaaa gactaaaaaa 1081
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ccatcaaaag aagtagaaga agatgaatac aaagctttct acaaatcatt ttcaaaggaa 1201
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attttatgtg taccacatc tgtccacgt ggtctgtttg acgaatatgg atctaaaaag 1321
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gtttcccgcg agactcttca gcaacataaa ctgcttaagg tgattaggaa gaagcttgtt 1501
cgtaaaacgc tggacatgat caagaagatt gctgatgata aatacaatga tactttttgg 1561
aaagaatttg gtaccaacat caagcttggg gtgattgaag accactcgaa tcgaacacgt 1621
cttgctaaac ttcttaggtt ccagtcttct catcatccaa ctgacattac tagcctagac 1681
cagtatgtgg aaagaatgaa ggaaaaacaa gacaaaatct acttcatggc tgggtccagc 1741
agaaaagagg ctgaatcttc tccatttgtt gagegaactc tgaaaaaggg ctatgaagtt 1801
atttacctca cagaacctgt ggatgaatac tgtattcagg cccttcccga atttgatggg 1861
aagaggttcc agaattgtgc caaggaagga gtgaagtctg atgaaagtga gaaaactaag 1921
gagagtcgtg aagcagttga gaaagaattt gagcctctgc tgaattggat gaaagataaa 1981
gcccttaagg acaagattga aaaggctgtg gtgtctcagc gcctgacaga atctccgtgt 2041
gctttggtgg ccagccagta cggatggtct ggcaacatgg agagaatcat gaaagcacaa 2101
gcgtaccaa cgggcaagga catctctaca aattactatg cgaagtcagaa gaaaacattt 2161
gaaattaatc ccagacaccc gctgatcaga gacatgcttc gacgaattaa ggaagatgaa 2221
gatgataaaa cagttttgga tcttctgtgt gttttgtttg aaacagcaac gtttcggta 2281
gggtatcttt taccagacac taaagcatat ggagatagaa tagaaagaat gtttcgctc 2341
agtttgaaac ttgacctga tgcaaagggt gaagaagagc ccgaagaaga acctgaagag 2401
acagcagaag acacaacaga agacacagag caagacgaag atgaagaagt ggatgtggga 2461
acagatgaag aagaagaac agcaaggaa tctacagctg aaaaagatga attgtaaatt 2521
atactctcac ctttggatc ctgtgtggag agggaaatgt aaatttacat ctttctttt 2581
tgggagagac ttgttttga tgccccctaa tccccctc cctgcactg taaaatgtgg 2641
gattatgggt cacaggaaaa agtgggtttt ttagttgaat ttttttaac attcctcatg 2701
aatgtaaatt tgtactattt aactgactat tcttgatgta aaatctgtc atgtgtataa 2761
aaataaaaaa gatcccaaat
```

FIG. 33

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Peptide Sequence

NP_003290

803 aa linear

Tumor rejection antigen (gp96) 1;
Tumor rejection antigen-1 (gp96);
glucose regulated protein, 94 kDa;
Endothelial cell (HBMEC) glycoprotein

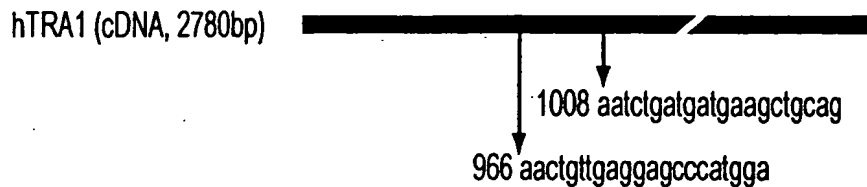
mralwvlglc cvlltfgsvr addevdvdgt veedlgksre gsrtdddevvq reeeaiql dg 61
lnasqirelr eksekfafga evnrmmklii nslyknkeif lrelisnasd aldkirli sl 121
tdenalsgne eltvkikcdk eknlhvtdt gvgmtreelv knlgtiaksg tseflnkmte 181
aqedgqstse ligqfgvgfy saflvadkvi vtskhndtq hiwesdsnef sviadprgnt 241
lgrgttitlv lkeeadsyle ldtiknlvkk ysqfinfpiy vwssktetve epmeeeeaaak 301
eekeesddea aveeeeeekk ptkkkvektv wdwelmdik piwqrpskev eedeykafyk 361
sfskesddpm ayihftaage vtfksilfvp tsaprglfde ygskkedyk lyvrrvfitd 421
dfhdmpkyl nfvkgvdsd dlplnvsret lqqhklkvi rklvrktld mikkiaddky 481
ndtfwkefgt niklgviedh sntrlakll rfqsshhtp itsldqyver mkekqdkiyf 541
magssrkeae sspfverllk kgyeviyte pvdeyciqal pefdgkrfqv vakegvkfde 601
sektkesrea vekefeplln wmkdkalkdk iekavvsqrl tespcalvas qygwsnmer 661
imkaqayqtg kdistnyyas qkktfeinpr hplirdmlrr ikededdktv ldavvlfet 721
atlrsgyllp dtkaygdrie rmlrlslnid pdakveepe eepeetaedt tedteqdede 781
emdvgtdede etakestaek del

FIG. 34

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siRNA-MEDIATED VALIDATION

siRNA MOLECULE DESIGN:



IN VIVO TARGET VALIDATION:

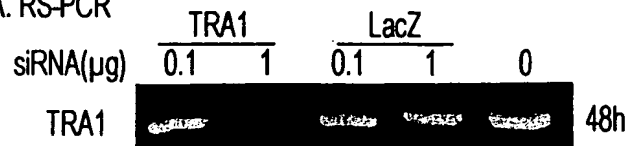
- MDA-MB-435 CELL PRODUCED XENOGRAFT TUMORS
- 3 REPEATED INTRATUMORAL ADMINISTRATIONS

IN VITRO PATHWAY ANALYSIS:

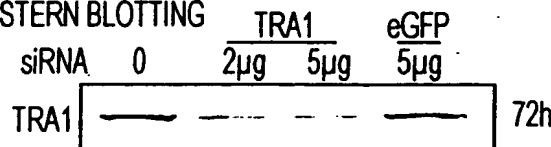
- siRNA TRANSFECTION IN MDA-MB-435 CELLS
- MEASURE APOPTOSIS ACTIVITY OF TREATED CELLS

FIG. 35

A. RS-PCR



B. WESTERN BLOTTING



siRNA-MEDIATED KNOCKDOWN OF TRA1 AT PROTEIN AND mRNA LEVELS IN CULTURED MDA-MB-435 CELLS

- RS-PCR DEMONSTRATED siRNA MEDIATED TRA1 KNOCKDOWN AT mRNA LEVELS IN MDA-MB-435 CELLS TRANSFECTED WITH VARIOUS DOSAGES OF TRA1-siRNA.
- WESTERN BLOTTING USING TRA1 ANTIBODY ILLUSTRATED THAT siRNA-MEDIATED KNOCKDOWN OF TRA1 AT PROTEIN LEVELS IN MDA-MB-435 CELLS TRANSFECTED WITH VARIOUS DOSAGES OF TRA1-siRNA.

FIG. 36

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ROLES IN APOPTOSIS

siRNA-MEDIATED ICT1025 KNOCKDOWN RESULTED IN
 INCREASED APOPTOSIS ACTIVITY IN MDA-MB-435 CELLS

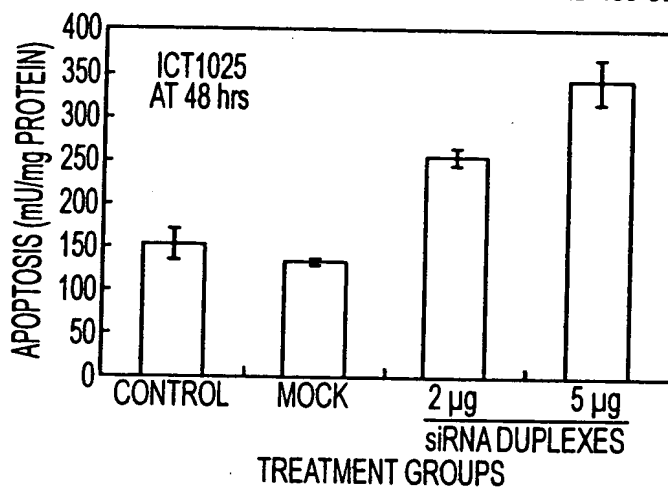


FIG. 37

EFFECTS OF 1025-siRNA ON CELL PROLIFERATION IN HT-29 CELLS
 AT 48 HOURS POST TRANSFECTION (MTT ASSAY)

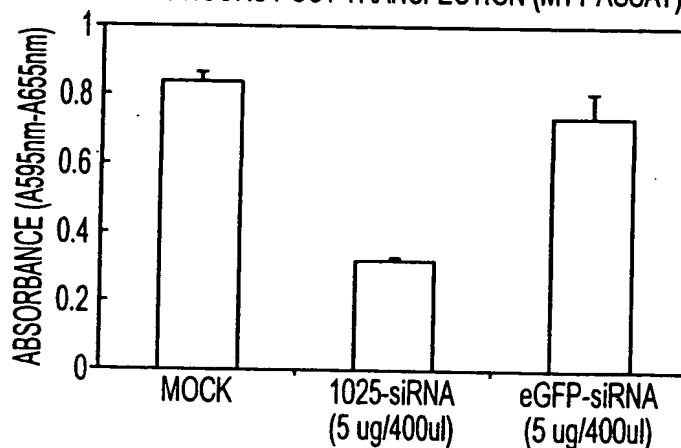


FIG. 38

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EFFECTS OF 1025-siRNA ON APOPTOSIS IN HT29 CELLS AT 48 HOURS POST TRANSFECTION (DNA FRAGMENTS IN CYTOPLASMA)

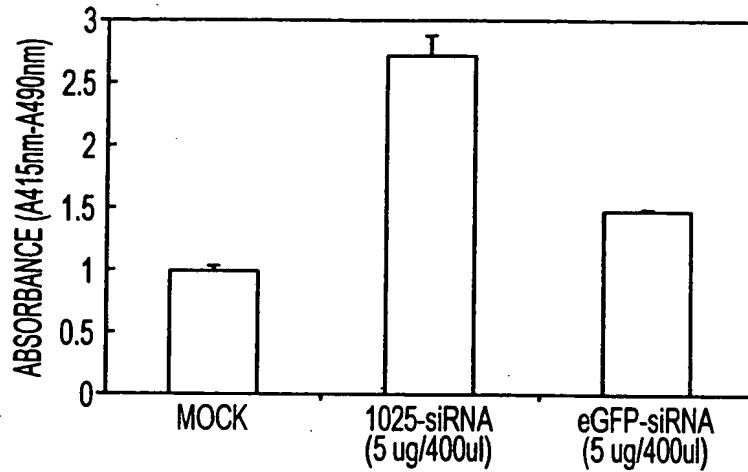


FIG. 39

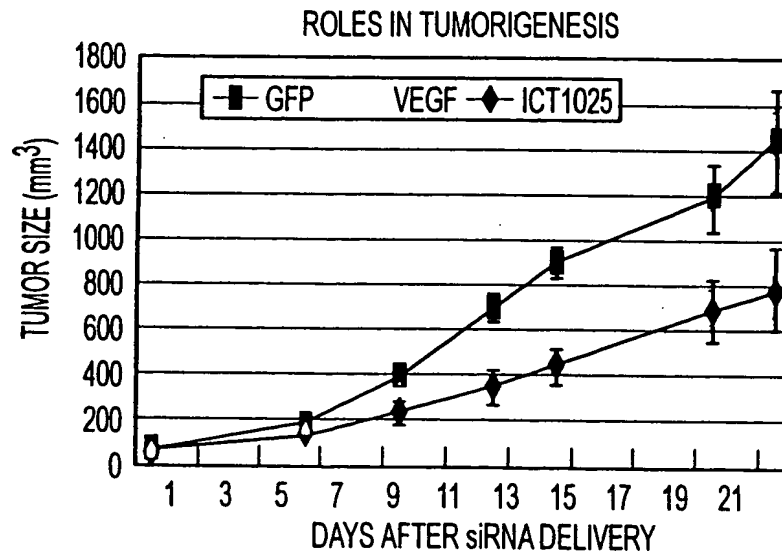


FIG. 40

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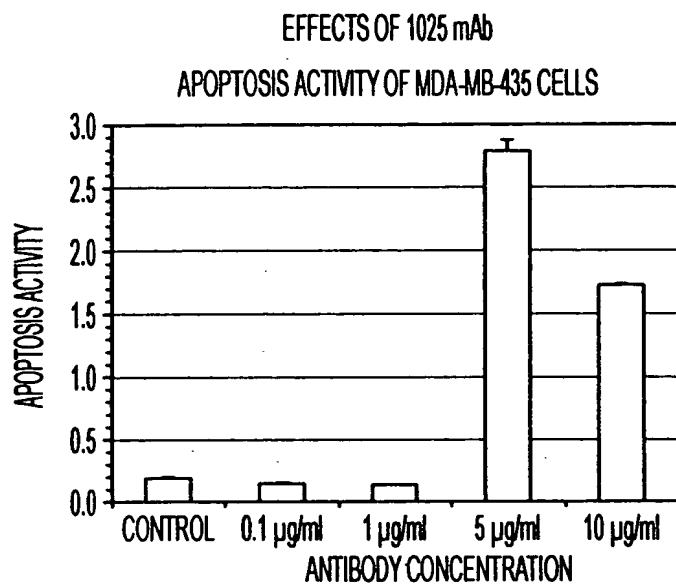


FIG. 41

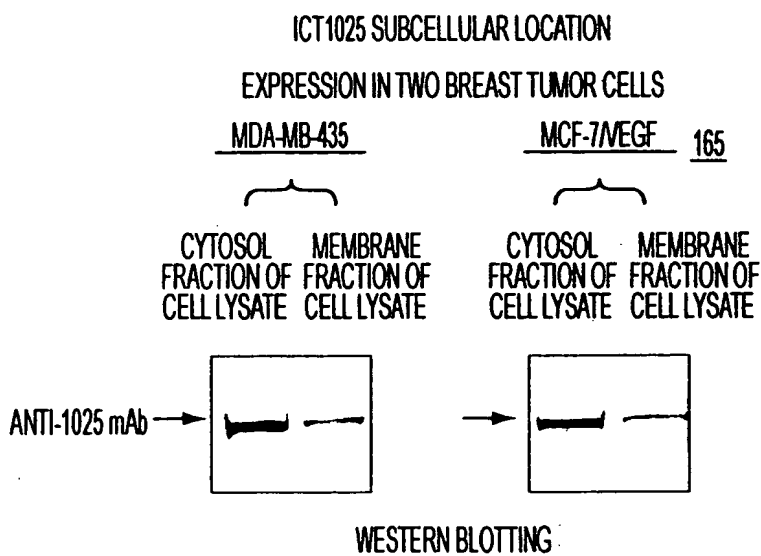


FIG. 42

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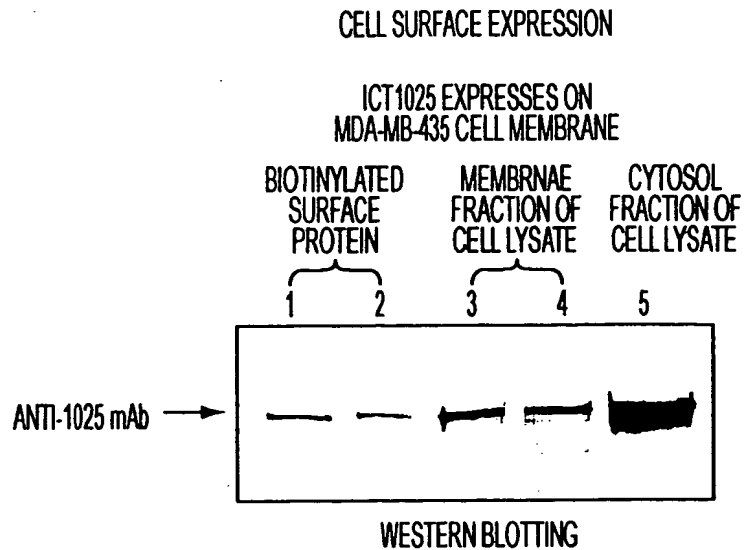


FIG. 43

VIRTUAL NORTHERN

TISSUE	EST DATA		SAGE DATA		EST DATA			SAGE DATA		
	NORMAL	CANCER	NORMAL	CANCER	NORMAL	CANCER	P	NORMAL	CANCER	P
ALL TISSUES	●	●	●	●	777 / 2078120	852 / 2025125	0.01	1007 / 3544388	1576 / 7876959	0
BONE	●	●			7 / 13882	48 / 43516	0.03			--
BRAIN			●	●				78 / 660821	806 / 3814713	0
CEREBELLUM			●	●				8 / 90885	67 / 252749	0
COLON	●	●			30 / 17811	64 / 149056	0			
KIDNEY	●	●			7 / 61080	23 / 73672	0.01			
LUNG	●	●	●	●	56 / 101826	51 / 165356	0	61 / 159917	22 / 122803	0
LYMPHORETICULAR	●	●			17 / 31613	13 / 77133	0			
MAMMARY GLAND	●	●	●	●	53 / 40986	37 / 82935	0	128 / 434551	276 / 1329651	0
MUSCLE	●	●			66 / 71281	15 / 36706	0			
PLACENTA	●	●			113 / 190017	13 / 39248	0.02			
PROSTATE	●	●			15 / 62027	41 / 60563	0			
UTERUS	●	●			4 / 32398	84 / 128509	0			

FIG. 44

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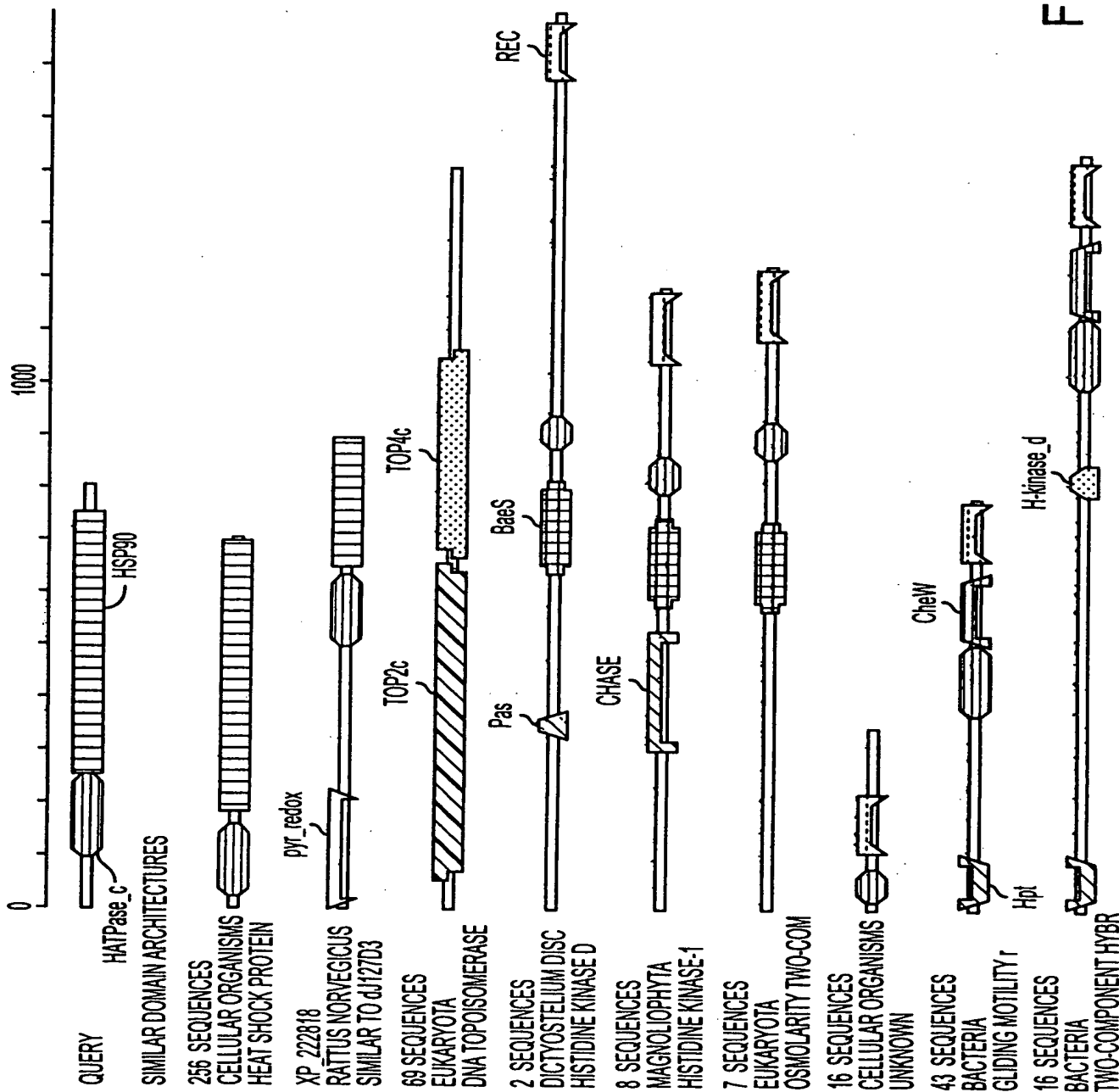


FIG. 45

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Human/Mouse Homology

human 1 mrllwvlglccvlltfgsvraddevdvdgtveedlgksregsrtdddevvqreeeaiqldglnasqirelreksekfafqaevnrmmklii
mouse 1 mrllwvlglccvlltfgsvraddevdvdgtveedlgksregsrtdddevvqreeeaiqldglnasqirelreksekfafqaevnrmmklii

human 91 nslyknkeiflrelisnasdaldkirlisltidenalqneeltvkikcdkeknllhvt dtgvgmtreelvknlg tiaksgtseflnkmt e
mouse 91 nslyknkeiflrelisnasdaldkirlisltidenalqneeltvkikcdkeknllhvt dtgvgmtreelvknlg tiaksgtseflnkmt e

human 181 aqedggstseligqfgvgfysaflvadkvivt skhnndtqhiwesdsnefsviadprgntlgrgttitlvkeeasdyeldtiknlvkk
mouse 181 aqedggstseligqfgvgfysaflvadkvivt skhnndtqhiwesdsnefsviadprgntlgrgttitlvkeeasdyeldtiknlvkk

human 271 ysqfinfpiyvwssktetveepneeaakeekesddeaaveeeeeekpktkkvektvwdwelmndikpiwqrpскеveedeykafyk
mouse 271 ysqfinfpiyvwssktetveepneeaakeekesddeaaveeeeeekpktkkvektvwdwelmndikpiwqrpскеveedeykafyk

human 361 sfskesddpmayihftaagevtfksilfvptsaprglfdeygskksdyiklyvrrvfitddfhdmmpkylnfvkgvvsddlplnvsret
mouse 361 sfskesddpmayihftaagevtfksilfvptsaprglfdeygskksdyiklyvrrvfitddfhdmmpkylnfvkgvvsddlplnvsret

human 451 lqghkllkvirkklvrktldmikkiadkyndtfwkefgtniklgviedhsnrtrlakllrfqsshhditsldqyvermekqdkiyf
mouse 451 lqghkllkvirkklvrktldmikkiadkyndtfwkefgtniklgviedhsnrtrlakllrfqsshhditsldqyvermekqdkiyf

human 541 magssrkeaesspfverllkkgyeviyltepveyqialpefdgkrfqnvakegvkfdesektkesreavekefepllnwmkdkalkdk
mouse 541 magssrkeaesspfverllkkgyeviyltepveyqialpefdgkrfqnvakegvkfdesektkesreavekefepllnwmkdkalkdk

human 601 iekavvsqrltespcalvasgygwsnmerimkaqayqtgkdistnyyasqkktfeinprhplirdmlrrikedddktvdlavvlfet
mouse 601 iekavvsqrltespcalvasgygwsnmerimkaqayqtgkdistnyyasqkktfeinprhplirdmlrrikedddktvdlavvlfet

human 681 atlrsgyllpdtkaygdriermrlrlsnidpdaaveepesstafitueedeqdedeemdvgtddeeeefakestaekdel*
mouse 681 atlrsgyllpdtkaygdriermrlrlsnidpdaaveepesstafitueedeqdedeemdvgtddeeeefakestaekdel*

FIG. 46

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hGRP94 MRALWVLGLCCVLLTFGSRADDEVDVDGTVEEDLGKSREGSRTVDEVVQREEEAIQLDGLNASQIRELREKSEKFAPQA 59
: : | |||
hHSP90 MPEEVHHGEEVEIIFAPQA 19

hGRP94 IVNRMMLIINSLYKNKEIFLRELISNASDALDKIRLISLTDENALSGNEELTVKIKCDKLNLLNVTDTGVGMTREELV 139
| : | ||| : | ||||| ||||| ||| | : | : ||| : || : :
hHSP90 EIAQLMSLIDNTFYSNKEIFLRELISNASDALDKIRYLSLTDPSKLD SGKLLKIDIIPMPQERILTLVDTGIGMTKADLI 99

hGRP94 MNLGTIAKSGTSEFLNTQTEAQEDGQSTSELIGQFGVGFYSAFLVADKVVIVISKHNNDTQHIWESDSNE.FSVIADPRGN 218
||| ||| | : : : : ||||| ||| : ||| : | : || : | : ||
hHSP90 NNLGTIAKSGTFAFMSAIQAGADIS....MIGQFGVGFYSAFLVAEKVVVIRNHNDDEQYAWESSAGGSFTVRAD.HGE 173

hGRP94 TLGRGTTITLVLKEEASDYLEIDTIGNLVKKYSQFINFPYVWSSKTETVEEFMEE.....EEAAKEEKELSDDEAA 290
: | | : | ||| : : || | ||| : || | ||| : | : | : | : | : | :
hHSP90 PIGMGTKVILHLKEDQTEYLEERRVKEVVIQHSQFIGYFPITLYLEKERIRIISDDEATEKKEEEDDKDCEEKRIEDV 253

hGRP94 VEEEEEE...KKPRTKKVEKTVWDWELMNDIKPIWQRPSKEVEEDEYKAPYKSFSKESDPHMAYIHFTAEGEVTFKSIL 366
: : || : || ||| : | : | ||| | : : || ||| : : : | : | : || : | : :
hHSP90 GSDEIDDSGKDMRKRTIQKIRIRYIQEELNNTKPIWTRNPDITQEYGEFYKSLTNDWEDHLAVRGFSVRGQLFFRALL 333

hGRP94 FVPTSAPRGLIDRYGSKKSDYIKLYVRRVIDTDDFKDMPKYLNFVKGVVDSDDLPLNVSRETQQHKLKLVIRKKLVK 466
| : | || || : || ||||| || : : || ||| : ||||| : || || | : |||| : : |
hHSP90 FIPRRAPFDLFEN..KKKKNNIKLYVRRVIMDSCDELIPEYLNFI RGVVDSLPLNISRIMLQQSKILKVIRKNTVKK 411

hGRP94 TLDMIKKIADDKYN.DTFNRTFGSNIKLGVTEDHSNRRLAKLLRFQSSHHTTDITSLDQYVERMKEKQDKIYFMAGSSR 525
| : : : | : || | | | : ||| : || : ||| : | : : || | |||| | || : | :
hHSP90 CLELFSSELAEDKENYKKRTYEAFSKNLKGIHEDSTNRRRLSELLRYHTSQSGDENTSLSYVSRMKETQKSIYYITGESX 491

hGRP94 KEAESSPFVERLLKKGYEVIYLTEPVCZYCIQALPEFCCMORFQIVAKDGVRTQSEKTSSEAEVEXEFEPLLNAMKDNA 605
: | |||| : | : || : ||| : ||||| ||||| | ||| : | | | | || | || : :
hHSP90 EQVANSAFVERVRKRGFYVVYMTPEIDECVQQLKEFDGKSLVSVTKEGLELPEDEEENKKMEESKAKFENLCNLMKEIL 571

hGRP94 LKDKIEKAVVSQRLTTSPCALVASQYGWSQNMIRIMKAQAYQGGNDISTNYYASQKKTFEINSPRHPLIRDMLKKIKED 695
| : || : | || | : | ||| : ||||| || : | || : || ||| || : : : : |
hHSP90 D.KKVEKWTISNLLVSSPCCIVTSTYGWSANMLRIMKAQAL...RDNSTMQYMKAKKHLEINPQHPIVETLRQKAEDEKN 647

hGRP94 DKTVLDLAVVLFETATLASGYLLPOTKAYQDRIEKLRLSLNIDPDAKVEEPEEEPEETAED.TTEDTRQDEDEEMDVG 764
|| | || : |||| | || : | || ||| : | | | | : : : :
hHSP90 DKA VNDLVVLLFETALLSSGFSLEDPOQTHSNRIYRMIXLGLG...IDEDE...VAAEFNAAVPDEIPPLEGDEDASRME 721

hGRP94 TDEEEETAKESIALKDEL 782
:
hHSP90 EVD 724

FIG. 47

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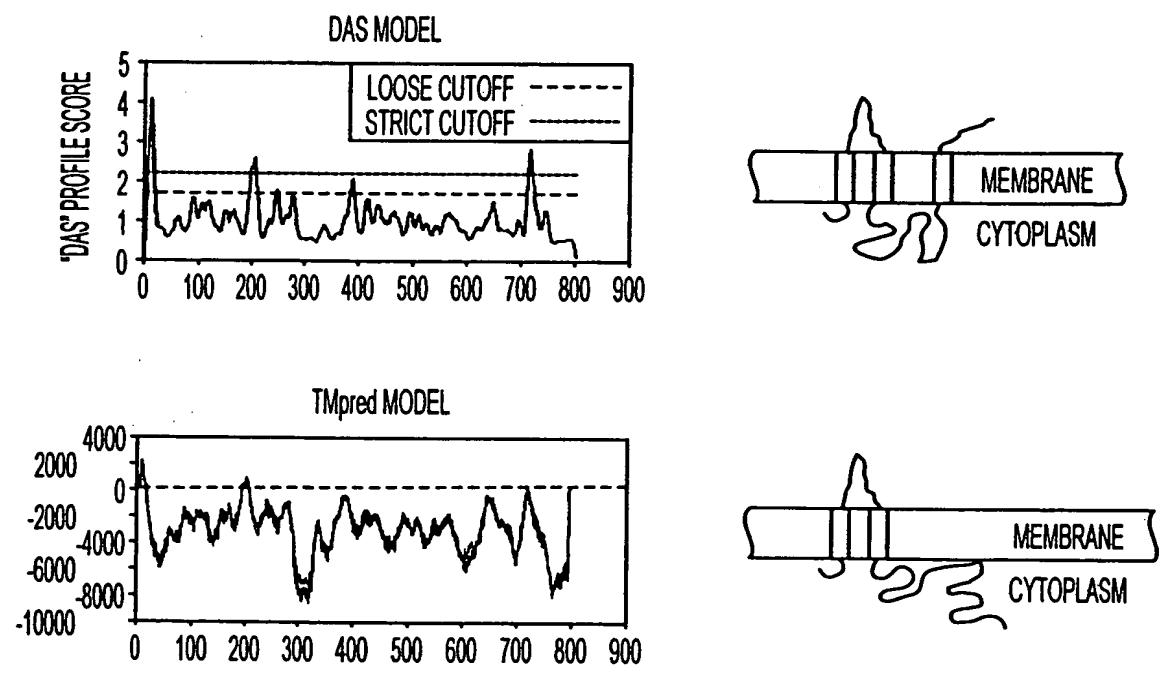


FIG. 48

PROKARYOTIC EXPRESSION VECTOR CARRYING ICT1025 cDNA

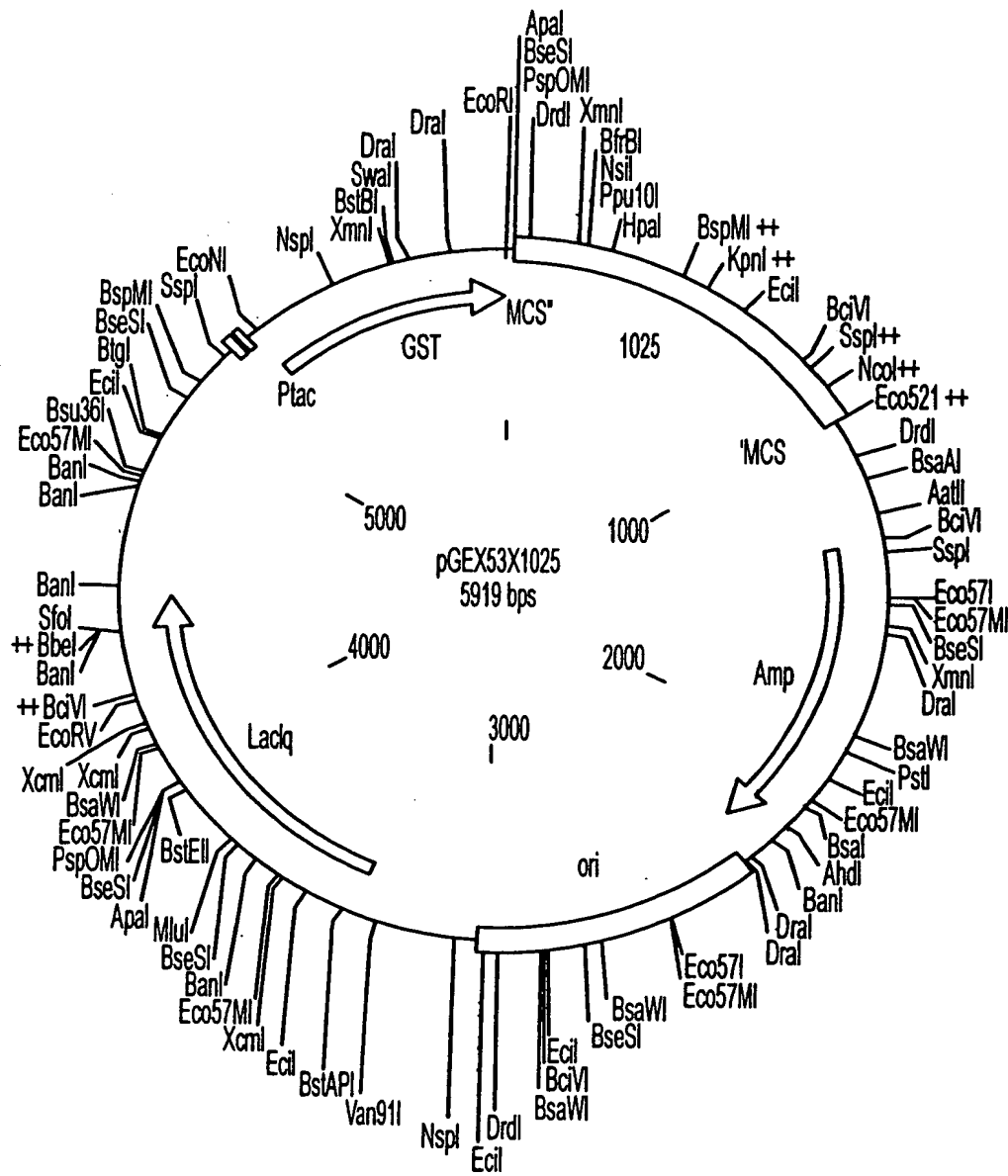


FIG. 49

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PROTEIN ISOLATED FROM BACTERIA

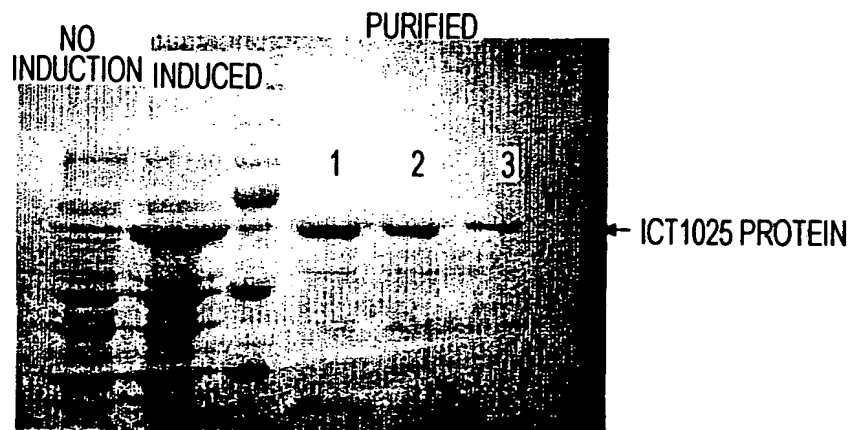


FIG. 50

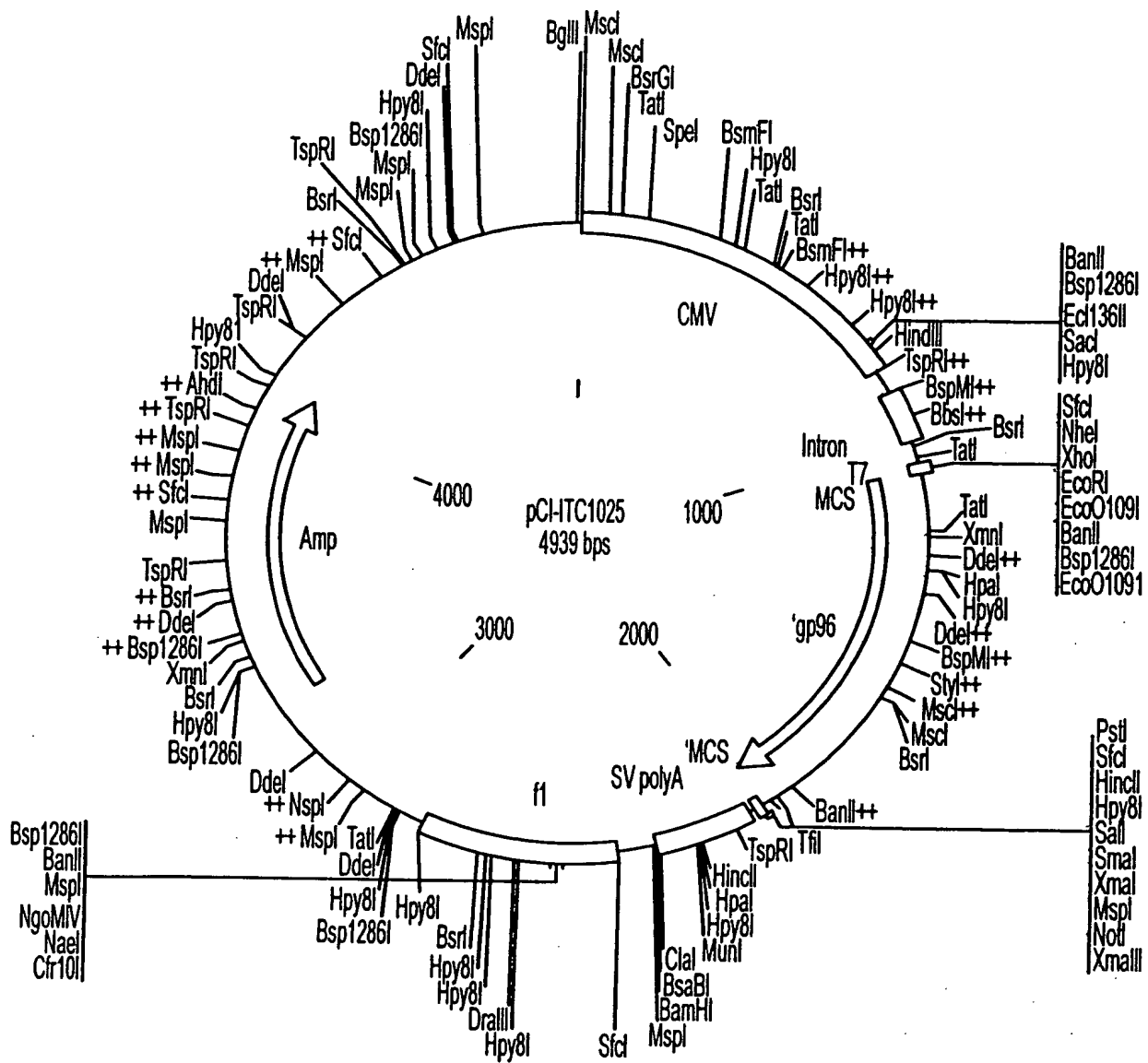


FIG. 51

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USER PARAMETERS AND SCORING INFORMATION	
METHOD SELECTED TO LIMIT NUMBER OF RESULTS	EXPLICIT NUMBER
NUMBER OF RESULTS REQUESTED	20
HLA MOLECULE TYPE SELECTED	A_0201
LENGTH SELECTED FOR SUBSEQUENCES TO BE SCORED	9
ECHOING MODE SELECTED FOR INPUT SEQUENCE	Y
ECHOING FORMAT	NUMBERED LINES
LENGTH OF USER'S INPUT PEPTIDE SEQUENCE	803
NUMBER OF SUBSEQUENCE SCORES CALCULATED	795
NUMBER OF TOP-SCORING SUBSEQUENCES REPORTED BACK IN SCORING OUTPUT TABLE	20

SCORING RESULTS			
RANK	START POSITION	SUBSEQUENCE RESIDUE LISTING	SCORE (ESTIMATE OF HALF TIME OF DISASSOCIATION OF A MOLECULE CONTAINING THIS SUBSEQUENCE)
1	425	MMPKYLNFV	1080.239
2	410	KLYVRRVFI	642.660
3	557	RLKKGYEV	257.342
4	203	FLVADKVIV	131.175
5	144	LLHVTDTGV	118.238
6	547	KEAESSPFV	106.738
7	639	RLTESPCAL	87.586
8	381	VTFKSILFV	76.863
9	3	ALWVLGLCC	41.234
10	6	VLGLCCVLL	36.316
11	189	SELIGQFGV	29.023
12	741	RMLRLSLNI	27.879
13	451	LQOHKLLKV	27.573
14	280	YVWSSKTET	24.895
15	259	LELDTIKNL	24.638
16	417	FITDDFHD	24.478
17	467	KTLDMIKKI	17.695
18	463	KLVRKTLDM	17.388
19	429	YLNFKGVV	17.053
20	197	VGFYSAFLV	16.564

FIG. 52

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SUGGESTED MODELS FOR TRANSMEMBRANE TOPOLOGY FOR ICT1025

—> STRONGLY preferred model: N-terminus inside

2 strong transmembrane helices, total score : 2962

from to length score orientation

1 3 19 (17) 2034 i-o

2 191 212 (22) 928 o-i

—> alternative model

2 strong transmembrane helices, total score : 2607

from to length score orientation

1 3 19 (17) 1929 o-i

2 191 213 (23) 678 i-o

FIG. 53

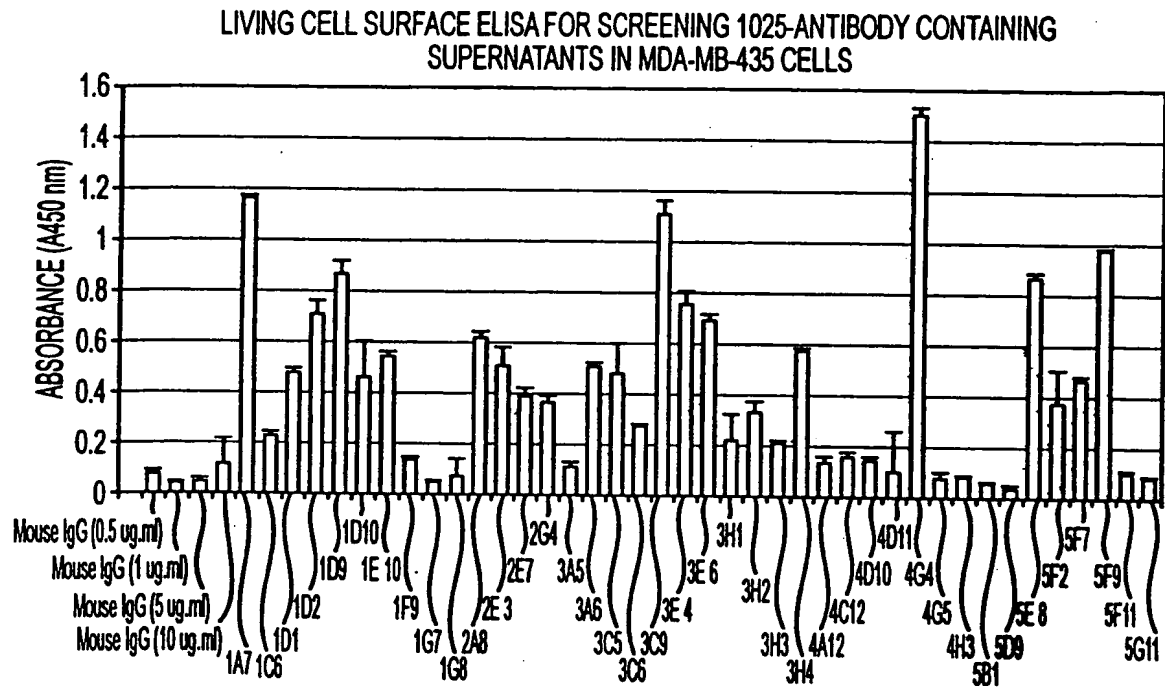
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"DAS" - TRANSMEMBRANE PREDICTION SERVER ICT 1025

POTENTIAL TRANSMEMBRANE SEGMENTS				
START	STOP	LENGTH	~	CUTOFF
6	18	13	~	1.7*
7	17	11	~	2.2
195	209	15	~	1.7*
197	206	10	~	2.2
247	248	2	~	1.7
384	390	7	~	1.7
710	723	14	~	1.7
713	719	7	~	2.2*

FIG. 54

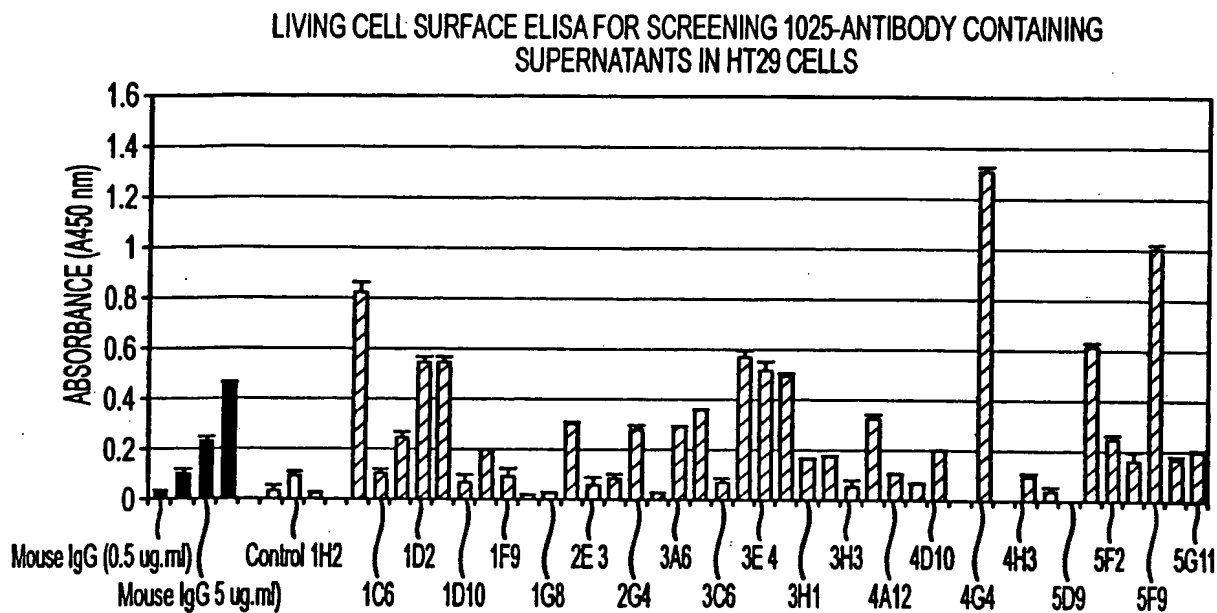
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SCREENING OF ICT1025 mAb FOR SURFACE BINDING ACTIVITIES IN BREAST TUMOR CELL

FIG. 55

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SCREENING OF ICT1025 mAb FOR SURFACE BINDING ACTIVITIES IN COLON TUMOR CELLS

FIG. 56

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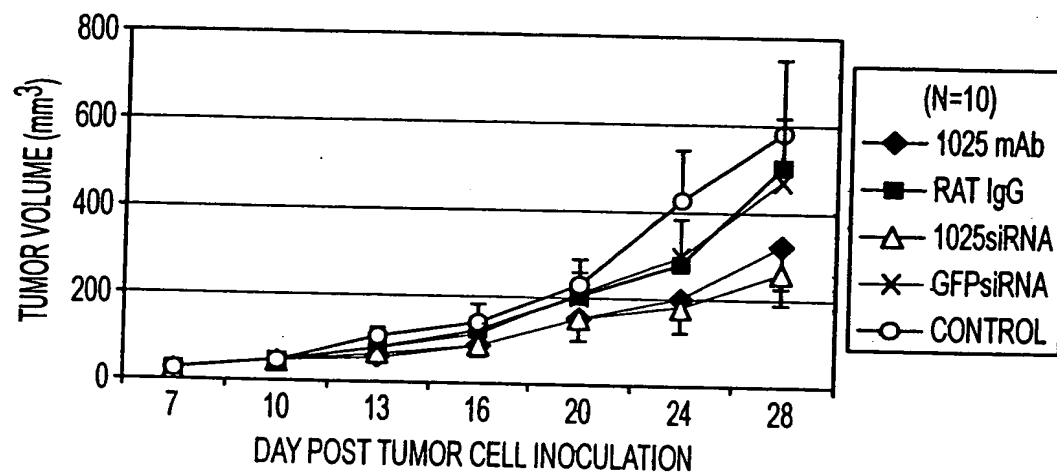


FIG. 57